

70.01
LW-2.00
6558
19099

GLEN CANYON ENVIRONMENTAL STUDIES

PHASE II

INTERIM PROGRESS REPORT

Submitted By

T. R. Angradi, R. W. Clarkson,
D. A. Kinsolving, D. M. Kubly, and T. Gamble
Research Branch
Arizona Game and Fish Department
2221 West Greenway Road
Phoenix, Arizona

**GCES OFFICE COPY
DO NOT REMOVE!**

Submitted To

Bureau of Reclamation
Glen Canyon Environmental Studies
P.O. Box 22459
Flagstaff, Arizona

Cooperative Agreement 9-FC-40-07940

ORIGINAL

August 1992

AQU 0317 -interpret B

Introduction

Dennis M. Kubly

Purpose of the Report

The purpose of this report is to provide the Bureau of Reclamation (Reclamation) with an update on research conducted by the Arizona Game and Fish Department (Department) subsequent to the Environmental Impact Statement (EIS) Technical Report of Angradi et al. (1992). That document presently is being edited for submission as a final report, so that it can be formally cited in the Glen Canyon Dam EIS.

Our reporting schedule in the Department's original GCES II Cooperative Agreement with Reclamation requires a second (draft) report by September 30, 1992, and a final (draft) report by December 31, 1992. Successive reports due in 1993 and 1994 are referred to as management reports. The expected content of these reports needs to be clarified by the GCES Program Manager for two reasons. First, the original Cooperative Agreement has been modified to allow for inclusion of additional efforts during the period of interim flows. Second, the Department's efforts are research based. Although these efforts will provide information to be used for future management of Colorado River wildlife resources, management reports *per se* would appropriately be generated by the Fisheries Branch, rather than the Research Branch.

Our present plans are that the September 1992 report will be more data intensive than the present report. It will incorporate analyses of all study elements to be treated in the December 1992 report and differ from that report primarily in the ending date for data incorporation. This approach will allow us to include recently collected data in the December report. It also will provide Reclamation with sufficient time to determine whether any additions or changes in scope are necessary, so that the Department can fulfill all allowable contractual obligations in this period of GCES Phase II.

Scope of the Report

The scope of this report, which generally covers the period November 1992-May 1993, emphasizes the following aspects: (1) delineation of new objectives or tasks incorporated into the research program; (2) description of new methods and analyses; (3) provision of preliminary results, and; (4) discussion of changes in the Colorado River ecosystem that have occurred during the period of interim flows.

The report covers the same study area of the Colorado River and its tributaries in the Grand Canyon region as that of Angradi et al. (1992), and it is arranged by similar study elements. A singular change is that native fish studies are divided between those occurring in the Little Colorado River and those located collectively in the Colorado River and other selected perennial tributaries.

Dam Operations During Interim Flows

Since August 1, 1991, Glen Canyon Dam has been operated according to interim flow criteria set forth by the Secretary of Interior. Minimum and maximum dam releases are restricted to 142 cms [cubic meters/sec (5,000 cfs)] and 566 cms (20,000 cfs), respectively, and

the former are further restricted to be not less than 227 cms (8,000 cfs) during the day. Daily ranges vary depending upon cumulative monthly water deliveries, but they may not exceed 227. Hourly increases are to be not greater than 71 cms (2,500 cfs), and hourly decreases are limited to 42 cms (1,500 cfs). Errors not to exceed 10% of these limits are allowed in recognition of difficulties in controlling releases. Exceptions have been granted for system emergencies, threats to human life, and economic considerations (forced power purchases). Expected effects from interim flow releases on geological, biological, and cultural resources of the Colorado River in the Grand Canyon region were projected in an Environmental Assessment conducted by the Bureau of Reclamation.

During the first nine months of the 1992 water year, recorded minimum flows at the USGS gaging station 2 km below Glen Canyon Dam varied between 146.2 cms and 205.9 cms (Table 1.1; note that flow records are still provisional). Months of higher minimum, mean, and maximum flows--December 1991, January 1992, and June 1992--were those of accordingly higher hydroelectric demand and production. This pattern is consistent with dam operations prior to the period of interim flows, excepting of controlled research flows, in that higher releases occurred during periods of higher electric demand. It differs in that lowest minimum releases in the past occurred during high demand winter months. The difference reflects constraints imposed on the daily minimum and range of flows during the interim flow period, i.e. minimum releases could not be less than 142 cms, and higher maximum flows require higher minimum flows. This observation is reinforced by monthly coefficients of variation, which are not noticeably higher for months of high hydroelectric output.

Graphs of daily minimum, mean, and maximum flows for three months during the 1992 water year--October 1991, January 1992, and May 1992 (Figure 1.1)--illustrate a phenomenon not discerned in the monthly analysis of flows. Weekend flows, and particularly Sunday flows, were considerably less than those occurring during weekdays. A one-way analysis of variance run on flow records taken at 15-min intervals showed that significant differences existed among days of the week for all three months. Scheffe's multiple range test confirmed that significant differences were largely between weekend days and week days (Table 1.2). These differences were most pronounced in October and least evident in January.

Literature Cited

- Angradi, T. R., R. W. Clarkson, D. A. Kinsolving, D. M. Kubly, and S. A. Morgensen. 1992. Glen Canyon Dam and the Colorado River: responses of the aquatic biota to dam operations. Prepared for the Bureau of Reclamation, Upper Colorado Region, Glen Canyon Environmental Studies, Flagstaff, AZ. Cooperative Agreement No. 9-FC-40-07940. Arizona Game and Fish Department, Phoenix, AZ. 155 pages.

TABLE 1.1.--Monthly flow (cms) statistics for the USGS gaging station 2 km below Glen Canyon Dam, October 1991-June 1992.

		Min	Max	Mean	SD	CV
1991	Oct	152.0	424.9	254.4	61.2	24.1
	Nov	164.8	403.8	282.0	67.1	23.8
	Dec	212.6	472.3	319.8	70.9	22.2
1992	Jan	233.4	491.4	356.8	71.0	19.9
	Feb	174.3	424.1	308.5	69.5	22.5
	Mar	161.0	444.8	268.7	61.4	22.8
	Apr	176.0	384.0	270.4	51.0	18.9
	May	146.2	400.4	272.9	51.9	19.0
	Jun	205.9	450.1	320.5	64.8	20.2

TABLE 1.2--One-way analysis of variance and Scheffe's multiple range test for day of week differences in flow (cms) at the USGS gaging station 2 km downstream of Glen Canyon Dam. Significant differences ($P < 0.05$) between days of week are indicated by *. Flow was recorded at 15-min intervals.

OCTOBER 1991								
F = 42.4384, $P < 0.001$								
Mean Flow		Sa	Su	Mo	Tu	We	Th	Fr
226.8	Sa							
224.4	Su							
262.2	Mo	*	*					
263.3	Tu	*	*					
265.9	We	*	*					
269.2	Th	*	*					
260.1	Fr	*	*					

JANUARY 1992								
F = 84.0339, $P < 0.001$								
Mean Flow		Sa	Su	Mo	Tu	We	Th	Fr
352.0	Sa							
290.8	Su	*						
376.1	Mo	*	*					
375.6	Tu	*	*					
354.5	We		*	*	*			
375.4	Th	*	*			*		
366.8	Fr		*					

MAY 1992								
F = 99.4674, $P < 0.001$								
Mean Flow		Sa	Su	Mo	Th	Fr	We	Tu
255.4	Sa							
232.4	Su	*						
280.4	Mo	*	*					
291.7	Tu	*	*					
289.9	We	*	*					
284.8	Th	*	*					
286.4	Fr	*	*					

Figure 1.1. Graphs of daily minimum, mean, and maximum flows for three months during the 1992 water year--October 1991, January 1992, and May 1992.

2. Ecosystem Processes and Lower Trophic Levels

Ted R. Angradi

The purpose of this chapter is to summarize the initial findings of studies of Glen Canyon ecosystem processes and lower trophic levels conducted in 1992 through mid June. In 1992, studies have focused on algal colonization and standing crop, particulate organic matter (POM) transport, and productivity of the amphipod *Gammarus lacustris*.

Previous studies of algal colonization indicated that accrual of algal biomass and chlorophyll *a* was influenced by flow fluctuations (Angradi et al. 1992). The objective of algal colonization experiments conducted in 1992 was to repeat the original field experiments under interim flows, and to expand the spatial scale of the experiments to include more of the Glen Canyon Reach. Monthly monitoring of periphyton standing crop begun in August 1991 was continued through the current period.

POM transport studies have indicated that concentration of POM exported from the Glen Canyon Reach does not greatly exceed that in water released from Lake Powell (Angradi et al. 1992, Angradi and Kubly, unpublished m.s.). In 1992, POM sampling was directed at exploring the longitudinal variation of FPOM concentration through Glen Canyon.

Several studies have shown that the amphipod *Gammarus lacustris* is an important component of the Glen Canyon ecosystem (reviewed by Blinn and Cole 1991). An important functional role of this organism appears to be the trophic transfer of periphyton primary production into trout biomass. Although the relative magnitude of this pathway remains to be confirmed, *Gammarus* is an important component of trout diets in Glen Canyon (Maddux et al. 1987). A study of the productivity of *Gammarus lacustris* was initiated in May 1992; data presented here are limited to distribution and abundance of *Gammarus lacustris* in Glen Canyon.

Methods

Periphyton Colonization

Accretion of periphyton chlorophyll *a* and biomass on cobble bars was determined using artificial substrates (tiles) cut from Navajo sandstone (Angradi et al. 1992). Tiles were deployed at -14 mile bar (2 km below the dam), -3.5 mile bar (20 km below the dam), and at Cathedral Wash (30 km below the dam, 5 km below the Paria River) in late February 1992. Tiles were placed in grids of 25 tiles each at three levels: <142 (< 5000 cfs), 199-227 (7000-8000 cfs), and 425 m³ s⁻¹ [cms] (15000 cfs); at Cathedral Wash the 425 cms level was omitted. At the sites in Glen Canyon, tiles were collected (*n*=3) at 10, 20, 30, 40, 60 and 100 days. At Cathedral Wash tiles were collected at 10, 20, 30, and 60 days.

The top and sides of each tile were scraped, and the resulting material was homogenized and subsampled twice. One subsample was ashed to determine ash-free dry mass (AFDM) of accumulated periphyton (Angradi et al. 1992); a second subsample was extracted in methanol for determination of chlorophyll *a* corrected for pheophytin (Tett et al. 1975).

Accretion of periphyton biomass and chlorophyll *a* on vertical cliff faces was determined using tiles mounted on boards bolted to the cliffs at 5-6 locations in Glen Canyon. Each vertical substrate set of eight tiles was mounted so that some tiles were never exposed, and some were exposed at known flows. In the first trial of the experiment, substrate sets (two each) were installed at 1.5 (on river left [RL]), 6.8 (RR), 14.0 (RR), 20.5 (RL) and 23.5 (RR) km downriver from the dam on March 1, 1992. Tiles were collected at 30 and 60 days and processed as described above. In the second trial tiles were redeployed, including an additional set 15.5 km (RL) from the dam, on May 11, 1992, and collected after 30 and 60 days (only results for 30 days are reported here).

Periphyton Monitoring

Methods used in the collection and processing of periphyton samples were identical to those described previously (Angradi et al. 1992). Samples were collected at -14 mile bar and -13.5 mile bar (3 km below the dam) from August 1991 through May 1992. No samples were collected in December 1991.

POM Sampling

Water samples were collected from penstocks at the dam and 1, 2, 3, 6, 9, 12, 15, 18, 21, and 25 km downriver. FPOM was obtained as described previously (Angradi et al. 1992). All samples were collected between 1300 and 1500 h during steady diurnal flows. Samples were collected on the first Tuesday and Wednesday of each month between February and May; samples were collected daily between May 31 and June 4, 1992. CPOM was not collected because it is time consuming to collect and it comprises <5% of the total transport of POM in Glen Canyon (Angradi and Kubly, unpublished ms.).

Gammarus productivity

In May and June, *Gammarus lacustris* was collected by a diver using a Hess Sampler (0.09 m²) fitted with a canvas cover. Samples ($n=8$) were collected at two sites (-14 mile, and -3.5 mile bars) and two levels (<142, 199-227 cms) from cobble and gravel substrates and along

permanent transects. In the laboratory, preserved samples (10% formalin) were sorted and all *Gammarus* were removed and counted.

Results and Discussion

Periphyton Colonization

At -14 mile bar, accretion of periphyton chlorophyll *a* and biomass was highest on the permanently inundated tiles (< 142 cms level; Figures 2.1, 2.2), but accretion of biomass was nearly as great on tiles at the 199-227 cms level. During the experiment, the tiles at the 199-227 cms level were virtually always inundated during the day (Figure 2.3). The difference in accretion rates on tiles from the two levels can be attributed to the effects of nighttime dewatering on the 199-227 cms level tiles. Tiles at the >425 cms level were always exposed and accumulated no periphyton.

At -3.5 mile bar, much less periphyton accumulated on the tiles than at -14 mile bar (Figures 2.1, 2.2). Tiles at the 199-227 cms level actually accumulated more chlorophyll *a* and biomass than the 142 cms tiles. Wave action from boats at this site may have obscured any effect of level on periphyton accumulation because permanently inundated tiles were subject to more sediment deposition than tiles in the wave-influenced fluctuating zone.

At Cathedral Wash, more chlorophyll *a* and biomass accumulated on the tiles in the fluctuating zone initially; after 60 days, more chlorophyll *a* and biomass had accumulated on the permanently inundated tiles. At the time that tiles were installed and at 20 and 30 days, the river was highly turbid due to sediment inputs from the Paria River. Turbidity was reduced during the second 30 days of the experiment (personal observation). Apparently, the effects of turbidity on light penetration interact with exposure effects due to the flow regime to determine periphyton colonization rates downstream of Paria River.

Accretion of periphyton chlorophyll and biomass on vertical substrates varied with exposure and distance from the dam (Figures 2.4, 2.5). The effects of exposure were most pronounced at the sites closest to the dam. In the first trial (Julian date 60-90, i.e., March-April), much more chlorophyll *a* and biomass accumulated on permanently inundated tiles than on exposed substrates. In the second trial (Julian date 132-162, i.e., mid May-mid June), accretion of chlorophyll *a* and biomass was similar for permanently inundated tiles and tiles at the 199-227 cms level. The difference in the amount of periphyton accumulated at the 199-227 cms level in the two trials is attributable to higher minimum flows in the second trial (Figure 2.3).

Much more periphyton accumulated on the vertical substrates closest to the dam in both trails. Standing biomass of periphyton on natural vertical surfaces adjacent to substrate sets showed the same trend. Differences in water velocity or orientation do not account for the longitudinal variation (Figure 2.6) in periphyton suggesting that periphyton may be nutrient limited in lower Glen Canyon.

Periphyton Monitoring

During interim flows, periphyton biomass in the permanently inundated channel initially decreased to a late fall minimum, and then increased in spring 1992 (Figure 2.7). Periphyton biomass in the zone of fluctuation (227 cms) increased to a winter maximum (February 1991) and decreased in spring 1992. The patterns of change in biomass of periphyton in the permanent and fluctuating zones were dissimilar because periphyton in the lower zone was undergoing seasonal variation while periphyton in the fluctuation zone was responding to flow variation. The decrease in biomass in the fluctuating zone in spring 1992 corresponds to a decrease in minimum flows (Figure 2.3). There is some evidence that the increase in minimum flow in late spring (May) is also detectable in the periphyton in the fluctuating zone (Figure 2.7). There was very little colonization of periphyton on natural cobbles above 425 cms.

POM sampling

FPOM concentration increased with increasing distance from the dam in April, May and June (Figure 2.8). The magnitude of the increase was small, as has been reported previously (Angradi et al. 1992). On June 2, a large increase (ca. twice ambient) in FPOM concentration was measured at the site 6 km below the dam. On the next two days an elevated FPOM concentration (ca. three-four times ambient) was measured at sites 15-18 km below the dam. As a result of a shift to a new release schedule at the dam, peak discharge on June 2 was higher than on any previous day since early March (Figure 2.3). These elevated peak flows partially flushed a large backwater located just upriver of the sample station (6 km). Flushed material was concentrated in large main-channel eddies further downriver (15-18 km). The effects of backwater flushing were short-lived and local; the FPOM concentration at Lee's Ferry on these dates was similar to dates when no backwater flushing occurred.

Gammarus productivity

Gammarus density varied among dates, sites, and levels. *Gammarus* density was higher at the -3.5 mile site, and at the permanently inundated level at both sites (Figure 2.9). Higher density in June than in May in the permanently inundated channel was probably due to recruitment into the smallest size classes (personal observation). Density in the fluctuating zone was lower in June than in May for unknown reasons.

Conclusions

Periphyton on cobble bars above the level of the permanently inundated channel appear to be very sensitive to flow regime. During interim flows, trends in the development of periphyton in this zone corresponded closely to changes in minimum flows. Complete recolonization of cobbles in the zone of fluctuation may be possible under interim flows, but this is doubtful since complete recolonization of permanently inundated substrates to natural ambient levels may require 100-300 days (e.g., compare Figures 2.2, 2.7). Periphyton in Glen Canyon appears to lack resistance to daytime desiccation is also sensitive to chronic nighttime exposure. Seasonal changes in minimum flows, especially on Sundays when low flows are minimal and most protracted, will determine the development rate of periphyton on cobble bars in Glen Canyon.

Evidence for nutrient limitation in Glen Canyon is still preliminary. It seems likely, however, that water quality and flow regime interact to determine periphyton development in Glen Canyon. In lower Glen Canyon, nutrient limitation may modify the magnitude of the exposure/desiccation effect on periphyton standing biomass.

POM in water released from Lake Powell appears to constitute the bulk of the POM exported from the Glen Canyon reach. This is additional evidence for the importance of Lake Powell forebay limnology on downstream ecosystem processes. For example, lake level determines the level at which water is withdrawn which may influence the particulate and dissolved nutrient content of released water.

Gammarus lacustris density varies among sites and degree of exposure. The higher density of *Gammarus* at the -3.5 mile site suggests that periphyton biomass, which is low at that site, is not the only determinant of *Gammarus* density. More likely, velocity and its effect on the availability of fine detritus exert a strong effect on *Gammarus* distribution and abundance in Glen Canyon.

Literature Cited

- Angradi, T. R., R. W. Clarkson, D. A. Kinsolving, D. M. Kubly, and S. A. Morgensen. 1992. Glen Canyon Dam and the Colorado River: responses of the aquatic biota to dam operations. Prepared for the Bureau of Reclamation, Upper Colorado Region, Glen Canyon Environmental Studies, Flagstaff, AZ. Cooperative Agreement No. 9-FC-40-07940. Arizona Game and Fish Department, Phoenix, AZ. 155 pages.
- Blinn, D. W., and G. A. Cole. 1991. Algal and invertebrate biota in the Colorado River: comparison of pre- and post-dam conditions. Pages 102-123 in Colorado River Ecology and Dam Management. National Academy Press, Washington, D.C.
- Maddux, H. R., D. M. Kubly, J. C. deVos, Jr., W. R. Persons, R. Staedicke, and R. L. Wright. 1987. Effects of varied flow regimes on aquatic resources of Glen and Grand Canyons. Final Report to GCES, Arizona Game and Fish Department. 291 pages.
- Tett, P., M. G. Kelly, and G. H. Hornberger. 1975. A method for the spectrophotometric measurement of chlorophyll *a* and pheophytin *a* in benthic microalgae. *Limnology and Oceanography* 20:887-896.

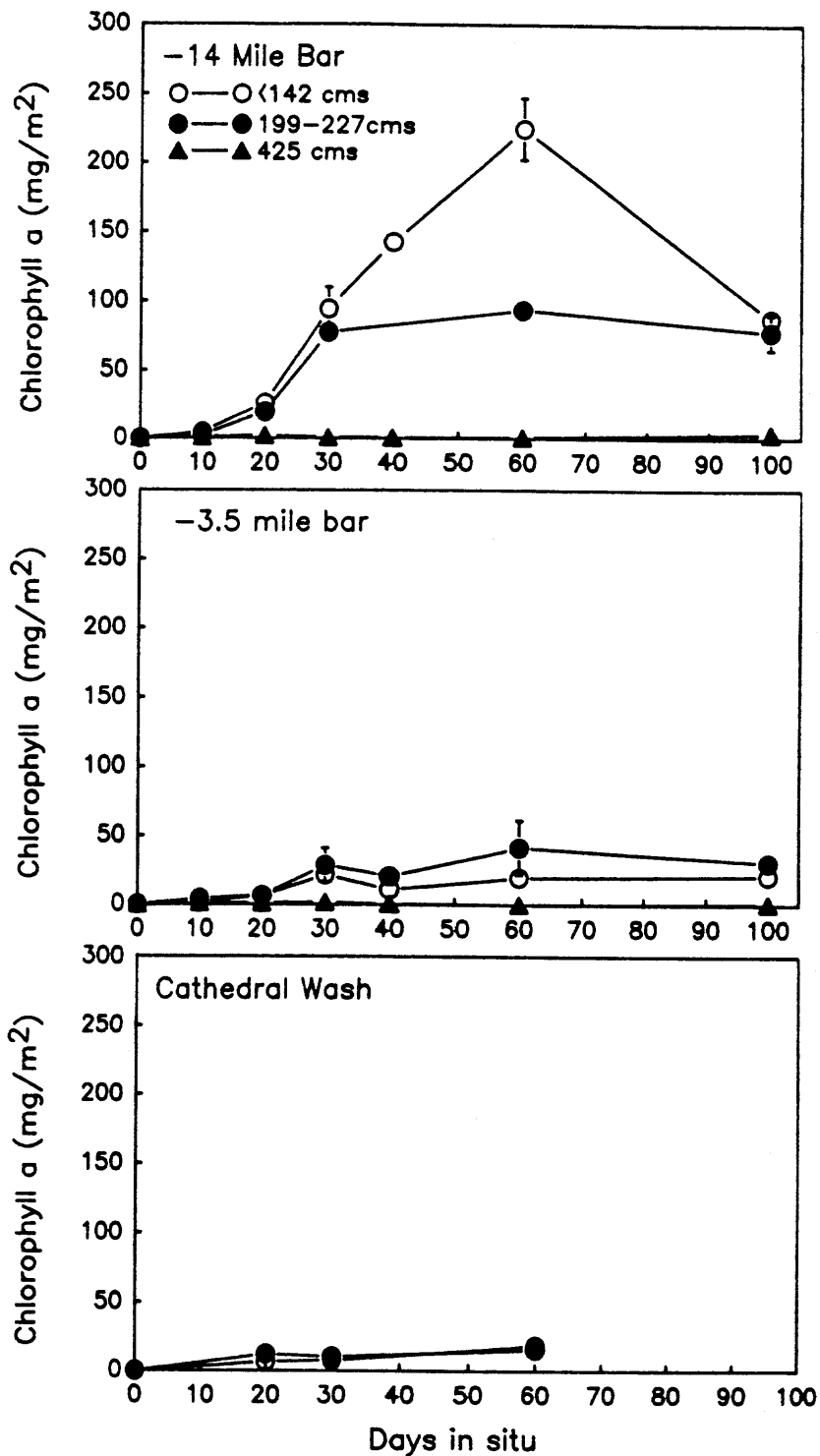


FIGURE 2.1.-- Accretion of chlorophyll *a* on sandstone tiles at three Glen Canyon sites in spring 1991 at three levels (cubic meters per second [cms]). Error bars are ± 1 SE of the mean.

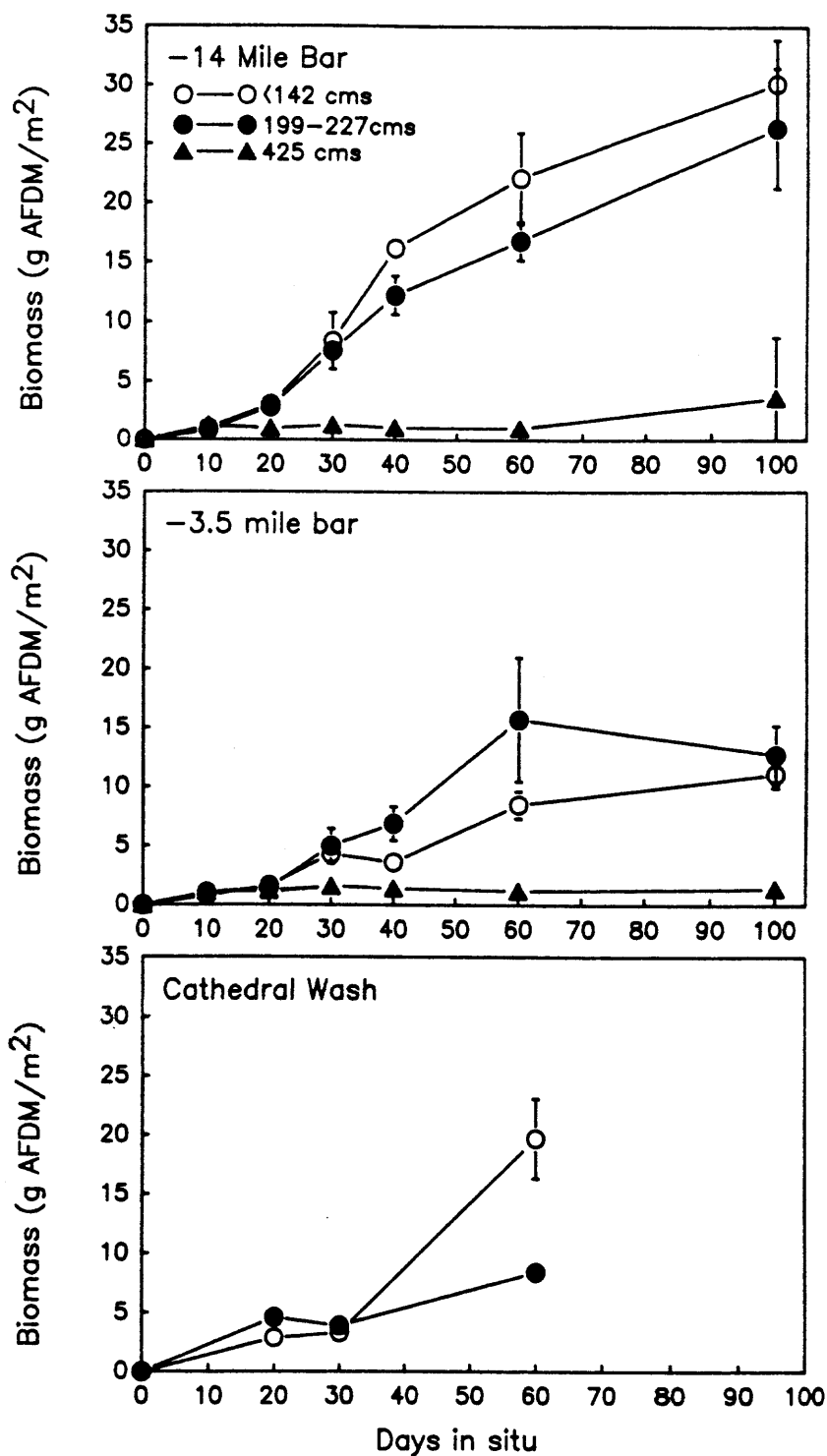


FIGURE 2.2.-- Accretion of biomass on sandstone tiles at three Glen Canyon sites in spring 1991 (cubic meters per second [cms]). Error bars are ± 1 SE of the mean.

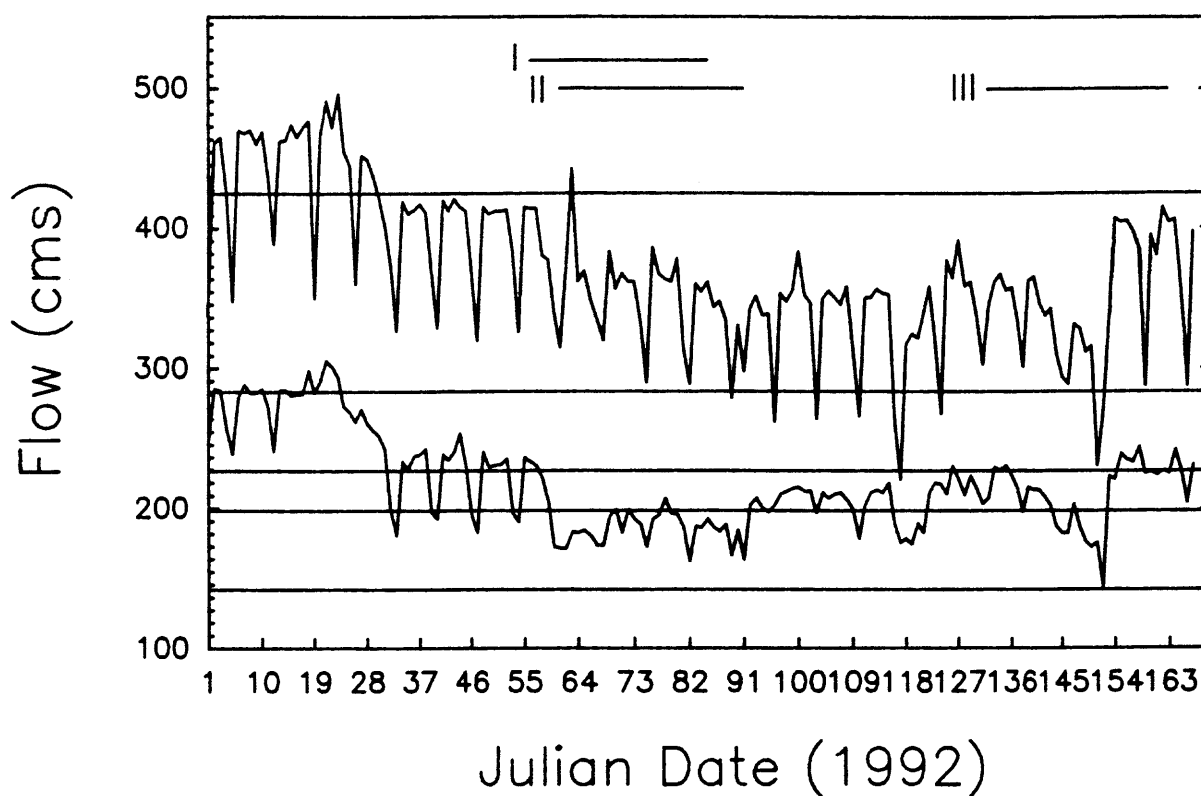


FIGURE 2.3.-- Minimum and maximum flows (cubic meters per second [cms]) during 1992 through June 15, 1992. Horizontal lines indicate significant levels (142, 199, 227, 285, 425 cms). Lines at the top of the figure indicate the time period of the cobble bar colonization experiment (I), and the two vertical substrate trials (II, III). Evenly spaced minima in maximum daily flow are Sundays. Data are from Glen Canyon Dam water release records.

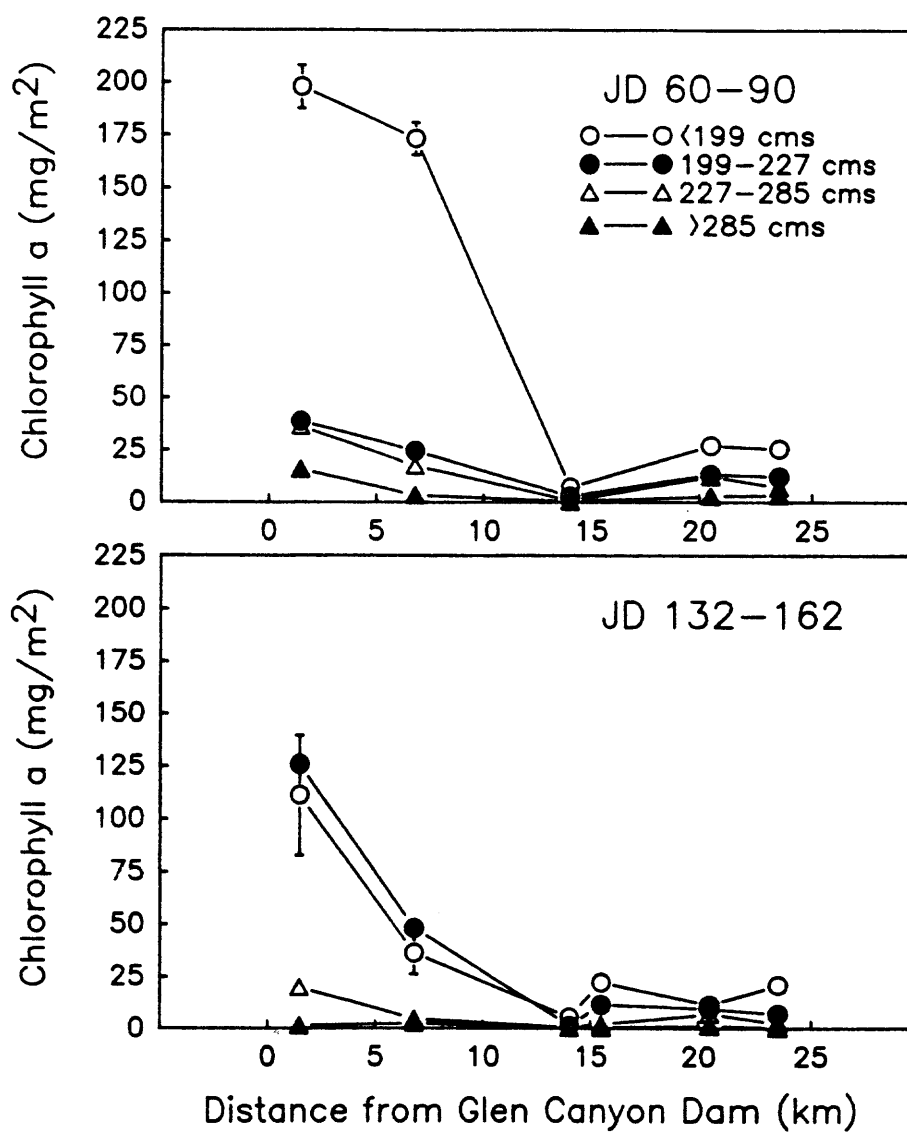


FIGURE 2.4.-- Accretion of chlorophyll *a* on vertical substrate sets in two experimental trials conducted from Julian date (JD) 60-90 and from JD 132-162 at four levels (cubic meters per second [cms]).

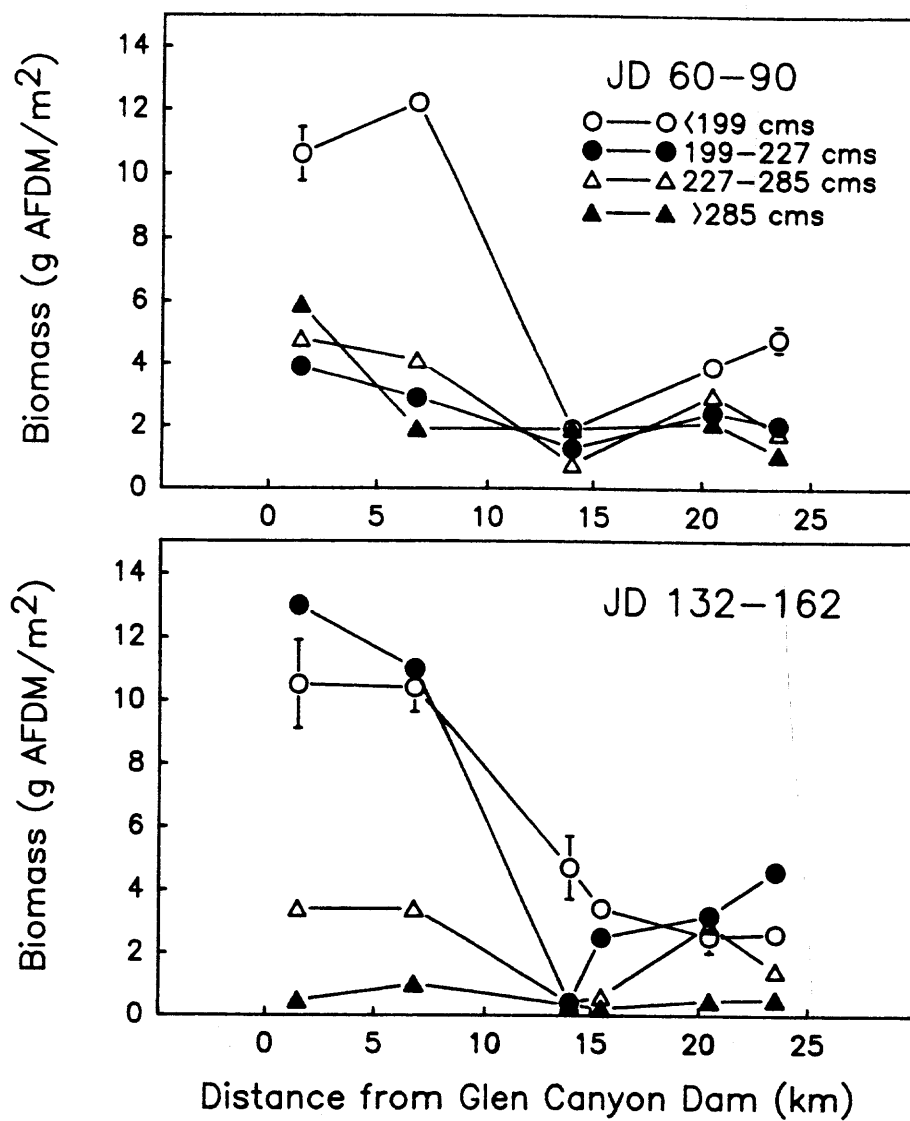


FIGURE 2.5.-- Accretion of biomass on vertical substrate sets in two experimental trials conducted from Julian date (JD) 60-90 and from JD 132-162 at four levels (cubic meters per second [cms]).

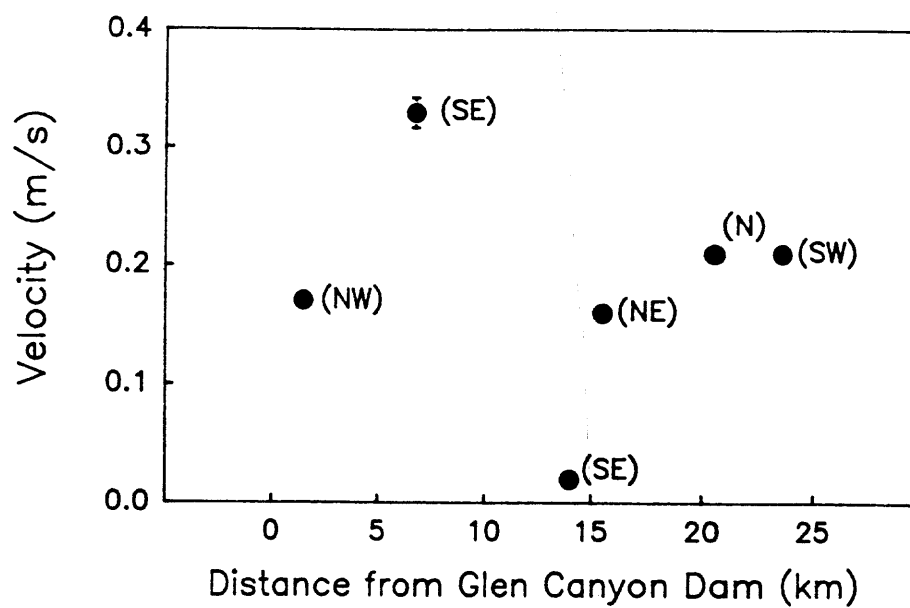


FIGURE 2.6.— Water velocity (± 1 SE) and (orientation) of vertical substrate sets in Glen Canyon. Velocity data were collected on June 10, 1992 from 1300-1500 h.

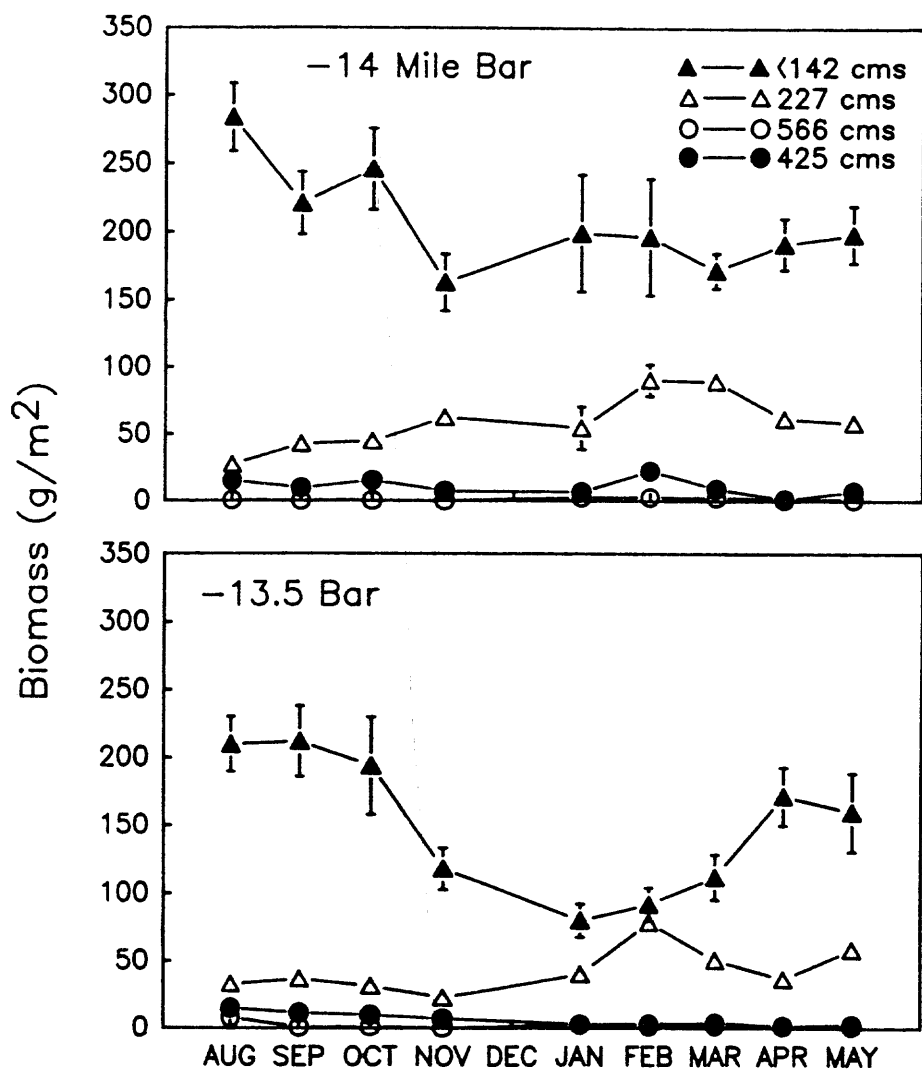


FIGURE 2.7.-- Accretion of biomass on natural cobbles at four levels (cubic meters per second [cms]) at -14 mile bar and -13.5 mile bar in 1991-1992. Error bars are ± 1 SE of the mean ($n > 10$).

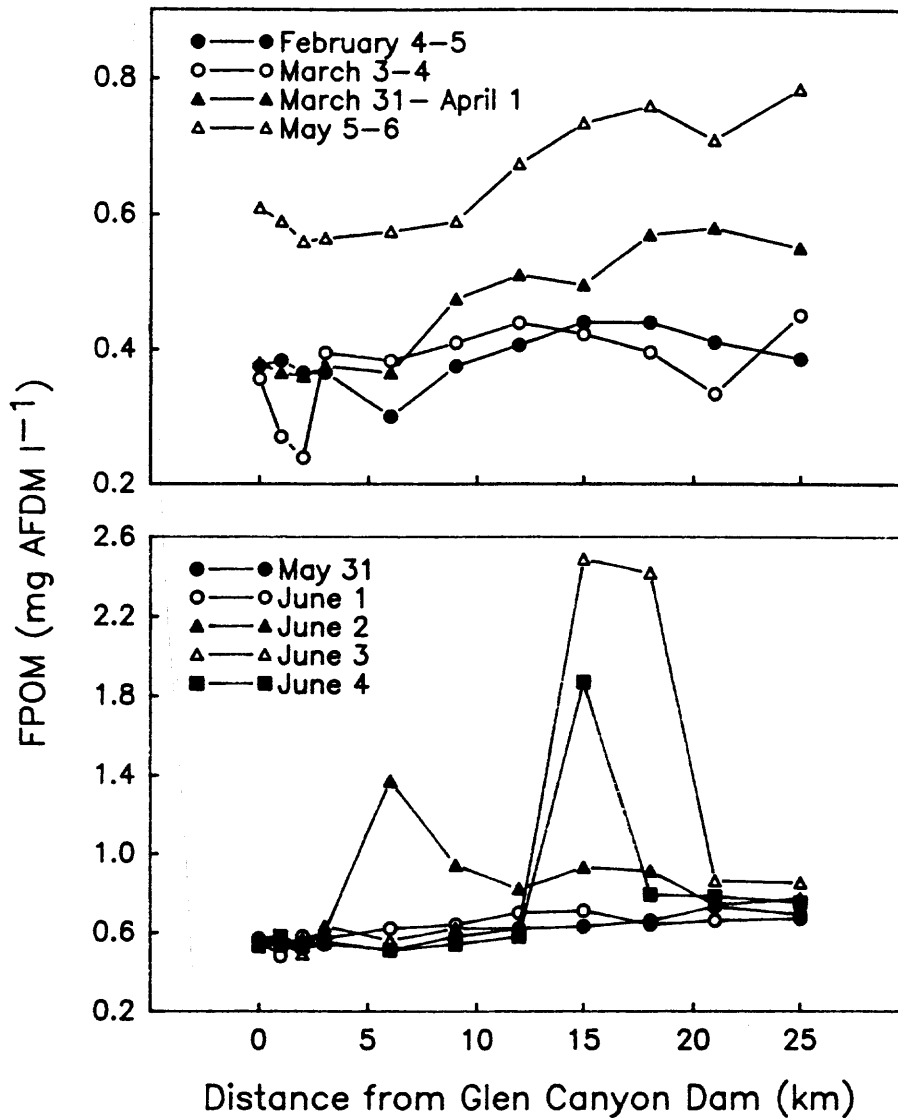


FIGURE 2.8.-- Longitudinal variation in mean FPOM concentration in Glen Canyon ($n=10$ except in June, $n=5$). Samples for 0 km were collected from Glen Canyon Dam penstocks. Error bars were omitted for clarity.

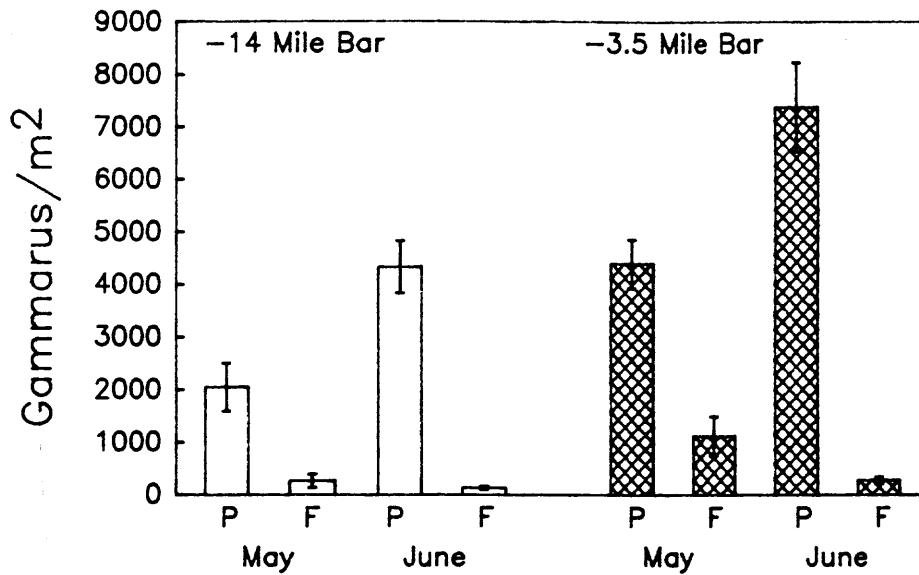


FIGURE 2.9.-- Mean (\pm SE, $n=8$) *Gammarus lacustris* density at two sites in Glen Canyon in May and June, 1992. P and F denote permanently inundated (<142 cms) and fluctuating (199-227 cms) zones, respectively.

3. Mainstem Colorado River and Other Tributaries

D. Alan Kinsolving

Methods

During the period from November 1991 through July 1992, six down-river trips took place resulting in a total of 89 field days. Trips utilized two different sampling protocols. On one type of trip, backwater and tributary sites were sampled as they were encountered. Sites were sampled at two levels of intensity. Opportunistic sampling was used to obtain a quick, fairly qualitative point in time characterization of a site. Intensive sampling was more quantitative, but still gave a point in time view. The second type of trip was designed to assess changes in fish abundance and habitat use across flow and diel cycles. One style of sampling, multi-day intensive, took place. On both trip types, chlorophyll and larval drift samples were taken. Trip dates and type of sampling are shown in Table 3.1.

At opportunistically sampled sites, fish were captured by making a single pass through the site using either a straight or bag seine (3-10 m long, 1-1.5 m high). The choice of gear depended on site size and topography, with bag seines used in larger, less structurally complex habitats. Additionally, dip nets were used to capture fish in areas where it was not possible to seine. Effort was recorded in square meters of water seined, and collected fish were identified, measured and enumerated following each seine haul. Unidentified fishes were preserved in 10% formalin for later identification in the lab. At each site the habitat type, dominant substrate, water temperature and site dimensions were recorded. Opportunistic sampling was generally used only for smaller sites, where it was not feasible to perform intensive sampling.

At intensively sampled sites, fishes were captured by two methods. In the backwater portion of the site, block nets (3.25 mm mesh) were emplaced as necessary in order to prevent fish escape from the site. The site was then electrofished using one or two hand held probes powered by a Coffelt VVP pulsator and a 5000 watt generator. Following electrofishing, the site was seined using either straight or bag seines (3-10 m long, 1-1.5 m high), depending on site topography and size. Electrofishing and seining continued until no additional fish were captured. During times of the year when very small (< 25 mm) fish were present, the site was also seined using a 0.84 mm mesh larval seine. In the mainstream and mainstream eddy portion of the site, fishes were captured in the same manner as for opportunistic sampling described above.

Because of the potential safety and fish health problems associated with electrofishing, it was temporarily discontinued during the April 1992 trip. We are currently evaluating our electrofishing gear and procedures and, if feasible, will continue the program in the future. After

electrofishing was discontinued, fishes were captured using only seines and dip nets. Normally, each site was seined at least three times, to allow the calculation of depletion estimates. However, it was frequently not possible to complete three seine hauls of the entire site, because the first pass would disturb fine sediments deposited on the bottom of the backwater, and subsequent attempts at seining would result in a large volume of fine sediment becoming entrapped in the net.

Each site was mapped using an alidade and plane table in order to create an accurate map showing wetted perimeter, contour lines for 25 cm, 50 cm, 100 cm and 150 cm depths, and areas of various substrates.

Water temperature and current speed were recorded at between 10 and 30 evenly spaced locations along the long axis of the backwater and at one location in the main river. Both measurements were taken at six tenths depth. Water temperature was measured to the nearest tenth of a degree C using a digital type K thermocouple thermometer. Current speed was measured to the nearest hundredth of a m/sec using a Marsh-McBirney meter. Dissolved oxygen (as percent saturation and concentration in mg/l) and water turbidity (in nephelometric turbidity units) were each measured at one location per habitat type. When water turbidity was greater than 100 NTU, it was necessary to dilute water samples prior to measuring turbidity. In those cases, 500 ml of the water to be measured was mixed with 500 ml of filtered water of known turbidity and measured. If turbidity of the dilute sample remained above 100 NTU, it was further diluted until turbidity was below 100 NTU. Actual sample turbidity was then calculated. Prior to using this technique, the formula for calculating turbidity of the actual sample from turbidity of the dilute sample was tested in the lab using a series of solutions of known turbidity.

Prior to shocking and seining the site, benthos, plankton and sediment samples were taken and preserved in 5-10% formalin for later lab identification and analysis. Benthos was sampled at two locations within each habitat type using a Petite Ponar dredge. Plankton samples were taken in each habitat type by pouring 30 l of water, collected from a single location, through 45 micrometer mesh plankton net. Sediment core samples were taken using a 50 cm³ minicore sampler.

Intensive multi-day sampling took place during trip 5 (11/1/91-11/18/91) in backwaters located at river mile 64.6 right, and 194.0 right, and Clear, Shinumo and Kanab creeks. Because the backwaters at mile 64.6 and 194.0 decreased in size during the period immediately after the beginning of interim flows, primarily because of the deposition of fine sediments that were not scoured away by high water, they were replaced by backwaters at river mile 68.0 left,

and 201.6 right, on trips 7 (2/19/92-3/5/92) and 9 (5/21/92-6/6/92). Clear Creek was also replaced by Crystal Creek on trips 7 and 9 because of the unsuitability of the Clear Creek area for camping. At each of these locations, from 30 to 50 minnow traps were deployed throughout the site. For backwater locations, traps were located in the mainstream, the inmainstream eddy, the return channel eddy and the backwater proper. At tributary mouth locations, traps were deployed in the mainstream and up the tributary as far as the 849.6 CMS (cubic meters per second) zone of fluctuation mark, with the emphasis on the current zone of fluctuation. Traps were checked approximately four to five times a day. Whenever possible trap checks took place during ascending water, stable high water, descending water and stable low water. During each trap check, current speed, depth, water temperature and substrate type were recorded for each trap as well as the species and length of fishes captured. Benthos, sediment and plankton samples were collected as described above at five to ten locations along a gradient of flow related influence ranging from locations that are submerged (or perpetually riverine) throughout the three day period to those that are submerged only briefly (or were influenced by the river only briefly). Each site was mapped using an ETM (Leitz or equivalent) supplied by GCES in order to accurately locate individual minnow traps and benthos sampling sites at a later time, and to preserve a record of topographic conditions at that time. Two data sondes (Hydrolab Corporation) were set to record pH, dissolved oxygen, conductivity, water depth and temperature at 20 minute intervals, were deployed at each site, in the mainstream and the tributary or backwater.

Qualitative tributary sampling took place during trips six, eight and ten. In general, tributaries were sampled in the area of the confluence (first 100 m of the tributary) and in an area either above a barrier falls (Shinumo Creek and Deer Creek) or approximately 1 km upstream from the confluence. Sampling took place in the manner described under opportunistic sampling above, with approximately 100-200 m² of effort devoted to both pool and riffle habitat in both the near confluence and non-confluence areas. Paria River sampling took place December 14th and March 29th 1992 at ten fixed sites located along the reach from the confluence to 3 km above the confluence. At each site approximately 50 m² of stream was seined using a 1/4" mesh seine.

Larval-drift nets 3 m long, with 750 μ m mesh net, 500 μ m mesh bucket and 0.25 m² opening were deployed in nearshore and tributary mainstem and tributary locations during trips seven, eight and nine. Current velocities were taken at the mouth of the net immediately after deployment and prior to retrieval. The mean of the two readings was used to calculate volume

of water filtered. Nets were deployed for periods ranging from 10 to 45 minutes, depending on the debris load and current speed. Because almost no larval fish are encountered in the mainstream drift, we increased the frequency of drift sampling beginning with trip nine to approximately six hours per day of drift netting effort. However, because this resulted in such a large number of samples, it was decided that the majority would be examined in the field, under a hand lens, for larval fish and then discarded.

Chlorophyll samples were collected by filtering 30 l of water through a 0.7 μ m glass fiber filter. Following filtration, the filter paper and residue were frozen in liquid nitrogen. Chlorophyll samples were taken during trip seven at six locations along the river above the Little Colorado River (LCR). It was not possible to collect samples below that point, or on subsequent trips, because high sediment loads in the river caused filter paper to clog well before an adequately large sample of water could be filtered.

Results and Discussion

Intensive multi-day sampling in backwaters resulted in low catches for all species during both the late fall (trip 5) and early spring trips (trip 7), with an average of only 3.75 fish/day being captured. Catches were considerably higher during the late spring trip (trip 9) for almost all species in both backwaters that were studied. Catches were generally larger in tributary streams than in backwaters throughout the period. However, on trip 5 no fish were captured in either Kanab or Clear Creek. As was noted for backwater sites, catches for all species in tributaries were generally largest during trip 9. Because effort data has not been fully analyzed, it is not possible to present these data in terms of catch per unit effort. However, effort was approximately equal between sites and between trips. Catch data for multi-day intensive sites is summarized in Table 3.2.

Data collected at intensively and opportunistically sampled sites during the same period showed similar seasonal patterns (Table 3.3), with catches lowest during the mid winter trip (trip 6) and highest during the mid-summer trip. Spatial patterns of fish distribution appeared to be similar to those found on earlier trips (Angradi et al 1992), with most native fish being rare or absent above the confluence with the LCR, and most abundant in areas immediately below the LCR and below National Canyon, in the lowermost portions of the study reach. Some notable exceptions to this were the absence of humpback chub in reach 50 (National Canyon to Diamond Creek), with the exception of two adult (> 200 mm) fish captured on hook and line at the mouth

of Havasu Creek, and an apparent increase in the number of fathead minnows encountered in reach 30 (LCR to Bright Angel Creek).

Length frequency data for all species collected during trips 5 through 8 and for humpback chub collected during trips 5 through 10 were examined. They are difficult to interpret. This is in part due to the selective nature of our sampling strategy, which does not target fishes larger than 150 mm. It is also due to low catch rates for all species throughout the winter and early spring. However, it appears that none of the species regularly encountered grew during the winter and spring months. It also appears that length frequency distributions for fishes captured in the mainstream are considerably broader and lack a distinct size peak for a given year class. This is logical if, as hypothesized (Angradi et al 1992), many fish enter the mainstream only after spending a portion of their early life in tributary streams, where conditions result in more rapid growth. Length frequency data for humpback chub are shown in Figure 3.1. Because data for other species was only examined for trips 5 through 8, and sample sizes were small during that period, those data are not shown.

Larval drift sampling did not result in the capture of any larval fish in the mainstream Colorado during this period. In the tributaries, four unidentified suckers were captured in Kanab Creek on 6/2/92. In addition, large numbers of larval fish were observed and taken in minnow traps during this same period. Because adult bluehead suckers were spawning at the time, it is probable that they were bluehead sucker larvae. No larval fish were captured during drift sampling in other tributaries (Crystal and Shinumo creeks). Because of the apparently small number of fish that enter the mainstream Colorado River drift and the large volume of water in which they are diluted, it will be necessary to increase the frequency and magnitude of mainstream drift sampling in the future.

Literature Cited

- Angradi, T. R., R. W. Clarkson, D. A. Kinsolving, D. M. Kubly, and S. A. Morgensen. 1992. Glen Canyon Dam and the Colorado River: responses of the aquatic biota to dam operations. Prepared for the Bureau of Reclamation, Upper Colorado Region, Glen Canyon Environmental Studies, Flagstaff, AZ. Cooperative Agreement No. 9-FC-40-07940. Arizona Game and Fish Department, Phoenix, AZ. 155 pages.

TABLE 3.1.--Number and types of samples taken and sites studied on trips five through ten.

Trip Number	Trip dates	Number of Sites			Benthos Samples Taken	Sediment Samples Taken	Plankton Samples Taken
		Multi-day Intensive	Intensive	Opportunistic			
5	11/1-11/18/91	5	0	0	39	43	48
6	1/5-1/19/92	0	15	13	33	56	28
7	2/19-3/5/92	5	0	0	30	22	15
8	4/12-4/26/92	0	32	11	60	60	60
9	5/21-6/6/92	5	0	0	24	24	7
10	6/21-7/3/92	0	26	13	50	50	49

TABLE 3.2.--Fish capture data for multi-day intensive sampling.

Location and Date	Flannemouth Sucker	Bluehead Sucker	Humpback Chub	Speckled Dace	Fathead Minnow	Plains Killifish	Rainbow Trout
Trip 5 (Nov, 91)							
Backwater, Mile 64.60 Right	0	0	8	2	5	0	0
Clear Creek	0	0	0	0	0	0	0
Shinumo Creek	1	0	0	256	0	1	0
Kanab Creek	0	0	0	0	0	0	0
Backwater, Mile 194.0 Right	0	0	0	10	2	0	0
Trip 7 (Feb/March, 92)							
Backwater, Mile 68.0 Left	0	0	0	0	10	4	0
Crystal Creek	0	0	0	35	0	73	0
Shinumo Creek	2	0	0	408	0	0	0
Kanab Creek	7	0	0	42	4	44	0
Backwater, Mile 201.6 Right	0	0	0	4	0	0	0
Trip 9 (May/June, 92)							
Backwater, Mile 68.0 Left	0	0	26	1	15	0	0
Crystal Creek	0	2	0	103	8	22	5
Shinumo Creek	0	18	0	501	3	0	7
Kanab Creek	10	22	0	203	20	6	0
Backwater, Mile 201.6 Right	18	0	0	1	9	0	0

TABLE 3.3.--Fish capture data for intensive and opportunistic sampling. Reach 20 is that portion of the Colorado River between Lee's Ferry and the Little Colorado River. Reach 30 is between the Little Colorado River and Bright Angel Creek. Reach 40 is between Bright Angel Creek and National Canyon. Reach 50 is between National Canyon and Diamond Creek.

Location and Date	Flannemouth Sucker	Bluehead Sucker	Humpback Chub	Speckled Dace	Fathead Minnow	Plains Kilifish	Rainbow Trout	Common Carp	Red Shiner
Trip 6 (JAN '92)									
Reach 20	0	0	0	0	0	0	0	0	0
Reach 30	0	0	0	0	0	0	0	0	0
Reach 40	2	1	0	1	3	4	0	0	0
Reach 50	10	0	0	1	1	1	0	0	0
Shinumo Creek	0	0	0	3	0	0	0	0	0
Tapeats Creek	2	0	0	0	0	0	0	0	0
Deer Creek	0	0	0	0	20	0	0	0	0
Trip 8 (APR '92)									
Reach 20	4	0	0	0	0	0	3	0	0
Reach 30	30	7	22	6	134	17	0	0	0
Reach 40	4	3	7	8	1	0	4	2	0
Reach 50	229	80	0	289	158	8	13	26	0
Nankoweep Creek	1	0	0	15	0	0	2	0	0
Clear Creek	0	0	0	6	0	0	14	0	0
Bright Angel Creek	0	0	0	1	0	0	6	0	0
Crystal Creek	0	0	0	2	0	0	2	0	0
Shinumo Creek	0	0	0	12	1	0	1	0	0
Deer Creek	0	0	0	0	0	0	17	0	0
Trip 10 (JUN/JUL '92)									
Reach 20	4	1	0	17	0	0	11	0	0
Reach 30	11	9	13	22	124	2	0	0	0
Reach 40	73	99	6	57	88	6	0	2	1
Reach 50	234	95	0	252	502	5	1	4	0
Bright Angel Creek	0	0	0	0	0	0	1	0	0
Pipe Creek	0	0	0	44	0	0	6	0	0
Crystal Creek	0	0	0	4	0	3	0	0	0
Kanab Creek	3	0	0	30	10	1	0	0	0

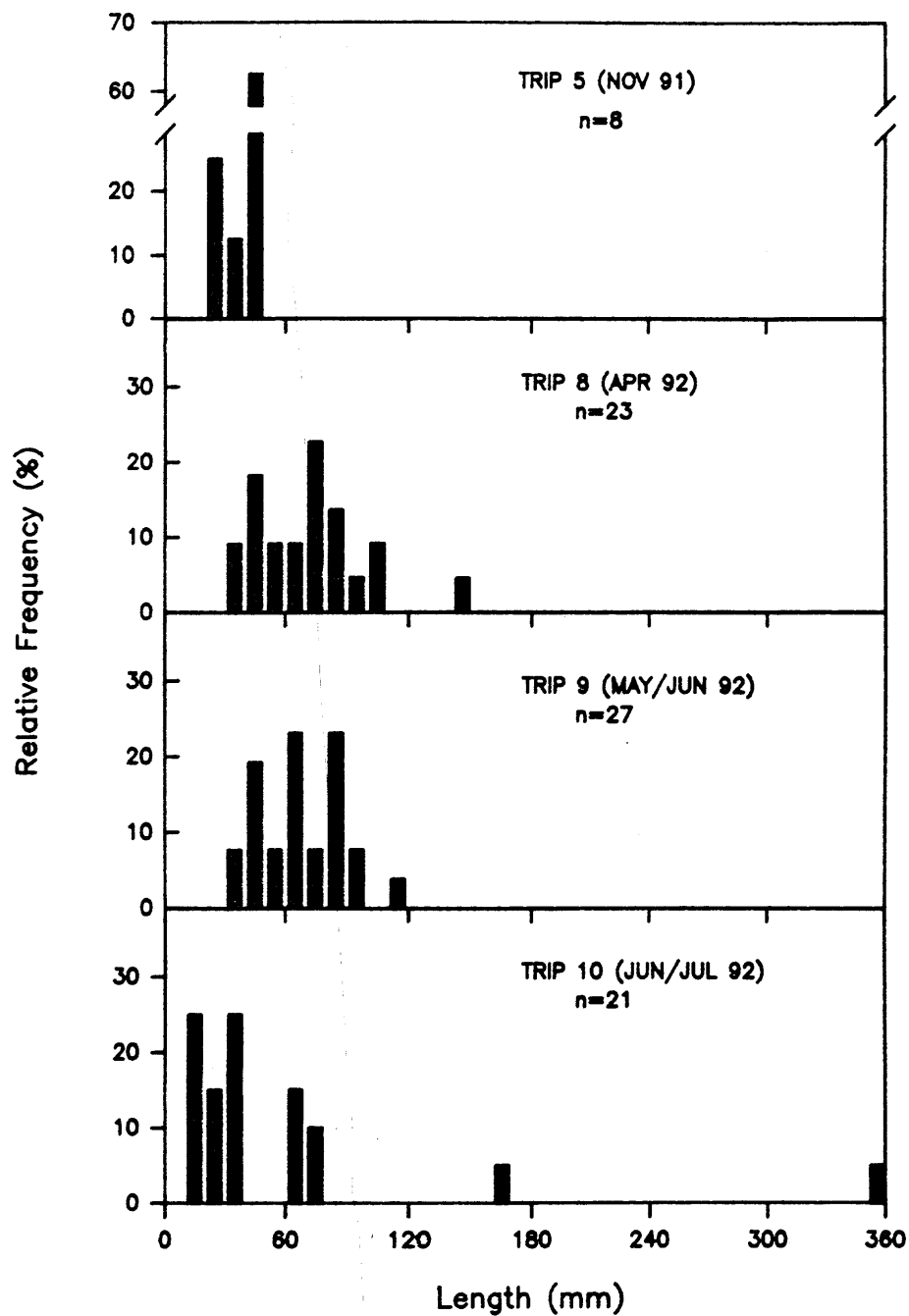


FIGURE 3.1.-Length frequency distributions for humpback chub captured in the Colorado River. No humpback chub were collected during trips 6 and 7.

4. Native Fishes - Little Colorado River

Robert W. Clarkson

This chapter updates research on humpback chub (*Gila cypha*) and other native fishes in the Little Colorado River (LCR) from November 1991 through May 1992. This period of research continued tracking growth and population status of the 1991 cohort of native fishes in the LCR, and refined and redirected research procedures to more fully describe native fish early life histories, especially for larval stages. New research activities were initiated on the 1992 age class of native fishes. Standardized hoop net monitoring of adult and subadult humpback chub was also accomplished during May 1992.

Spawning sites of many southwestern native fishes in riverine environments are in areas of swift current (Minckley 1973). However, these sites are often poor producers of zooplankton (Hynes 1970), a major food source for many larval fishes (Minckley 1973, Snyder 1990), and larvae have difficulty contending with swift currents (Harvey 1987). Thus larvae must disperse to other areas to satisfy their energy requirements (Corbett and Powles 1986, Tyus and Haines 1991). Prior research has indicated that downstream transport of native fish larvae via drift in the LCR is a common phenomenon, and may be of major significance in the ecology of this life stage (Angradi et al. 1992).

Observations of larval distributions in shoreline areas of the LCR in 1991 indicated that many seemingly suitable habitats were never occupied, despite physical similarity to occupied sites. It is unknown on what basis larvae populate suitable rearing habitats once they have moved from the site of hatching. Since larval movements are limited by current velocities, hydraulic variables may dictate whether a given habitat is accessible from the mainstem (Floyd et al. 1984, Harvey 1987, 1991). Other features such as algal or zooplankton densities, substrate types, cover attributes, presence of predators, or physical-chemical variables such as temperature may ultimately determine suitability of accessible habitats. Recent AGFD LCR studies have attempted to define nearshore availability of larval habitats and habitat utilization patterns.

The temporal and spatial distribution of larvae in nearshore habitats immediately following spawning may identify major spawning reaches if spawning sites are localized. If there is fidelity to spawning sites across years, such locality information could be used to more precisely define sites of egg deposition for future studies of reproduction. Studies in 1992 have attempted to establish the relationships of temporal and spatial occurrence of larvae in nearshore habitats to adult spawning activity.

Dispersal mechanisms of larvae may include drift or active movements via swimming along shorelines. Techniques that monitor movements of fishes among nearshore habitats can provide further insight into larval dispersal mechanisms (Brown and Armstrong 1985, Harvey 1991). Recent research has attempted to determine movements among habitats, including passive transport (drift) of larvae, from spawning sites and larval nearshore habitats to post-larval midchannel habitats.

Little is known about behaviors of YOY native fishes of the Grand Canyon region. Knowledge of potential temporal or spatial segregation of species among habitats and among age classes within species, schooling, agonistic interactions, selection for cover, foraging behaviors, etc., is important to fill life history information gaps and for conservation purposes. Research in 1992 has continued to document behaviors in terms of time activity budgets, microhabitat use, and food habits.

Methods

Discharge patterns of the LCR in 1992 (Figure 4.1) and the sampling problems such flows created prevented the undertaking of many of the methods detailed below. They are outlined to provide the reader with a complete view of the integrated study plan that is in place to ascertain early life history patterns of native fishes in the LCR.

Propagation of Humpback Chub

Some studies on native fishes of the Grand Canyon cannot be completed in the field and will be conducted under controlled conditions in a laboratory or other off-site environment. Two attempts at field-fertilization of humpback chub eggs were made in the LCR in late March and late April following methods of Hamman (1982, personal communication). Detailed methods of the April trip are presented here.

Adult fish were collected in hoop nets, weighed, measured and PIT-tagged, with males and females held separately in live cars. Ripe females were manually stripped of eggs into a shaded plastic spawning bowl and milt from two or three males added. Sperm diluent was then added, followed by bentonite solution. After 60 seconds, eggs were washed, and total egg volumes (± 1 ml) and weights (± 0.01 g) recorded. Two sample counts were made to provide an estimate of the number of eggs/g and ml.

Unripe females were injected intraperitoneally up to three times at 24-h intervals with 1 mg/ml concentrate carp pituitary extract at a dosage of 4 mg/kg body weight. When eggs were readily expressed, the above procedure was applied to pituitary-injected fish.

Eggs were then transferred to one of four hatching groups. One group of naturally ripe eggs was placed in a covered floating Heath incubator tray in the mainstem LCR, and the other group placed in a covered cradle-shaped floating egg basket in Salt Trail Canyon outflow. Eggs from pituitary-injected fish were split into similar hatching treatments.

Eggs were held in these trays variously for up to three days prior to transport by helicopter and truck in oxygenated bags to AGFD Bubbling Ponds Hatchery at Page Springs, Arizona. Eggs were then ionically and thermally tempered over a 24-h period and placed in Heath incubators and hatching jars at Bubbling Ponds. Egg samples were taken from all treatments at 12 to 24-h intervals and preserved in 5% formalin.

Larval Fish Longitudinal Surveys

Quantification of the temporal appearance and spatial distribution of larval native fishes in the LCR was accomplished by conducting longitudinal surveys of nearshore habitats between Atomizer Falls and the mouth during the reproductive season. Shorelines (both banks) were walked once or twice weekly beginning in April, and occupation of nearshore slackwater habitats by larval fishes within contiguous 100 m reaches was recorded as presence/absence. At base flow LCR nearshore waters were shallow and clear enough to allow direct observation of small fishes occupying these habitats. When turbid conditions were present, sweeps with fine-meshed aquarium nets and seines were performed.

When possible, fishes were identified in the field with the aid of portable 45X stereo dissecting microscopes. Collections were also preserved for lab identifications, stomach contents, and otolith analyses. Larval collections were made from 100 m reaches at 0.5 km intervals to avoid oversampling, unless distributions dictated otherwise.

Larval Fish Habitat Availability

U.S. Fish and Wildlife Service (FWS) perpendicular-to-flow transects found within every fifth 100 m reach (both banks) beginning at river kilometer 0 (mouth) were measured for current velocity, depth, substrate and habitat complexity features to assess the river-wide availability of larval fish habitats. Depths were recorded to ± 1 cm. Current velocity in areas exceeding 5 cm in depth were measured at 0.6 depth to ± 0.01 m/s using a Marsh-McBirney electromagnetic

flow meter. In shallower areas, neutral-bouyancy beads were drifted and timed over a distance of 10 cm. Substrates were classified to categories listed in Table 4.1. Habitat complexity features included depths >0.5 m, surface turbulence, turbidity, ledges, substrates larger than gravel (64 mm), undercut banks, overhanging vegetation, instream vegetation, and woody debris.

Measurements were taken at 10 cm, 25 cm, and at 25 cm intervals from shore thereafter until a current velocity was recorded that exceeded 0.2 m/sec. Previous observations of larval habitat use indicated that this velocity should be sufficient to include all nearshore larval habitats. The habitat availability procedure was performed at both base flow and at higher flows, and will be repeated again after larvae or post-larvae have left the nearshore, slow-velocity habitats.

Habitats at each transect were subjectively categorized to peripheral pools (shoreline invaginations), vegetated shoreline margins (e.g. *Typha*, *Scirpus*, or *Phragmites*), non-vegetated shoreline margins, and springflow channels. Physical measurements within these habitat types will be compared to groupings generated from principal components analysis or other multivariate techniques.

Four 100 m study reaches were established for more intensive larval habitat availability measurements. Depth, current velocity, substrate, and habitat complexity features (as above) will be recorded along 2 m spaced perpendicular-to-flow transects at 10 cm intervals extending from 5 cm from shore to a point in the mainchannel where flow velocities exceed 0.2 m/s. This procedure will yield a total of 400 transects (50 per each of four reaches, both banks).

Larval Fish Habitat Use

Within each fifth 100 m reach (both banks) along the river between the mouth and Atomizer falls, when YOY fish were caught or seen a series of up to three perpendicular-to-flow transects were established for taking habitat use measurements (depth, substrate, current velocity, habitat features; as above). These transects were spaced no closer than 10 cm apart, and extended through the area inhabited by the fishes. Measurement intervals along each transect was 10 cm. The number of transects run depended on the size of the area inhabited by the fish; if the area was less than 20 cm wide then only two transects were established; if the area of utilization was 20 cm wide or more then three transects were established. Transects were spaced evenly in larger habitats so that the area occupied by fishes was adequately sampled.

All nearshore habitat types occupied by larvae within the four reaches identified for intensive habitat availability measurements will be measured for depth, current velocity and

substrate at cell midpoints using a 20 cm square grid system (400 cm² grids) of 12 grids. Four peripheral pools (two occupied, two unoccupied) and two shoreline margin habitats (one occupied, one unoccupied; lengths not to exceed 3 m) within each of the four reaches (both banks) will be randomly selected and fish abundance and distribution, water temperature, algal and zooplankton densities will be estimated using the gridded sampling regime. Habitat features will be enumerated. Habitat cover features will be ranked at each sampling point, and water temperatures taken using a hand-held thermometer near sunrise and sunset to estimate thermal maxima/minima. Mean zooplankton abundance within the habitat will be estimated by pumping a measured volume (typically 8 l) of water into a plankton net. Algal abundance (as chlorophyll *a*) will be estimated in each grid using a mini-core sediment sampler and freezing the sample on dry ice for later laboratory spectrophotometry. 35 mm photographs of these gridded habitats will be taken to determine microhabitat use patterns. Frequency of observation will be once per hour for 24 h using a programmable camera with flash. Species identification, especially with early larval stages, is problematic with this technique. Where possible, larval collections will be made immediately following observation periods. Data from occupied habitats and grids will be compared to unoccupied habitats and grids, and to habitat availability data using the Manly (1974) selectivity index and graphical techniques (e.g. Thomas and Taylor 1990). Frequency distributions and discriminant analysis will be used to compare habitat parameters between used and unused habitats (Christensen 1985).

Habitat suitability index curves (Bovee 1981) will be generated for the seven parameters quantified above. The relationships between mean frequency-of-use of cells and physical-biological parameters in those cells will be evaluated using multiple linear regression or discriminant function analysis.

Direct observations of habitat use of YOY fishes will be supplemented with collections from larval seines, fine-meshed dip nets, and other methods. In this manner, larval fishes and other macroscopically unidentifiable life stages can be sorted in the lab and species associations determined.

Larval Fish Movements

In order to determine the mechanism(s) of longitudinal dispersal of larval fishes among nearshore habitats, larval traps similar to the design of Culp and Glozier (1989) were emplaced at inflow (upstream) and outflow (downstream) points of a subsample of occupied peripheral pools and shoreline margins. Traps were made from transparent plastic 500-1000 ml wide-

mouth bottles, with the central portion of the bottom and screw cap cut out, and fitted with a 500 μm -mesh screen funnel in the cap and a flat screen on the bottom.

Numbers of fishes present in each habitat at the start of each sampling period will be estimated visually at base flows. Four 100 m reaches (two in the vicinity of each camp where larvae are present) will be selected for these and other detailed analyses. A minimum of two shoreline margins and two peripheral pool habitats will be sampled within each of the four reaches (on both banks), staggered so that each reach is sampled once a week. Traps will be deployed and run at 6-h intervals encompassing a 24-h period. If possible following enumeration, trapped fishes will be released alive immediately above or below the trap site, depending on their direction of travel at the time of capture. Emigration rates may thus only be determinable from initial trap sets. Escape rates from traps will be estimated by placing a known number of larvae within traps and monitoring losses over time.

Two standard larval drift nets (3 m long, 0.25 m² opening, 750 μm mesh net, 500 μm mesh bucket) will be placed in deeper habitats, one nearshore adjacent to trap locations, and the other midchannel. Drift nets will be run at the same intervals as larval traps. Water volumes filtered will be estimated by measuring current velocities at the mouths of drift nets at the beginning and ending of each sampling period. Depths sampled will also be recorded.

Fish Behavioral Analyses

Time bound focal animal behavioral analyses (Altman 1974) will be continued in 1992 until sufficient sample sizes (~ 50 for each category) are obtained to characterize both species and species size class activities (general behaviors and vertical positioning in the water column). An individual fish will be observed for a period of 5 min and behavior changes recorded on audio tape. Behavioral categories will include feeding, swimming, schooling, chasing, being chased, hiding, and other. Cumulative time spent in each category will be transcribed from the tape recordings.

Vertical occupation of the water column will be similarly evaluated by recording movements among five zones: 1) in contact with the bottom (benthic); 2) lower one-third of water column but not in contact with bottom (lower pelagic); 3) middle one-third of water column (mid-pelagic); 4) upper one-third of water column but not at surface (upper pelagic), and; 5) at surface (surface). Observation periods will include early morning, mid-day, and early evening. If not identifiable visually, the observed fish will be collected for identification.

Fishes will be assigned to one of six groups based on ontogeny (Snyder 1981) and habitat use for among-group comparisons: 1) protolarvae (larvae characterized by undeveloped spines or rays associated with future median fins); 2) mesolarvae (larvae characterized by morphogenesis of distinct principle rays in the median fins; 3) metalarvae (larvae characterized by presence of full adult compliment of principle fin rays in the median fins and presence of pelvic fins or fin buds); 4) post-larvae in nearshore slackwater habitats, and; 5) post-larvae in mainstem habitats.

Samples of larval and juvenile native and introduced fishes were collected monthly for analysis of digestive tract contents. Relatively large collections were made of larval specimens unidentifiable in the field to better ensure an adequate sample size for each species. Monthly collections of 10 specimens of YOY humpback chub > 30 mm TL were taken for these analyses, split between the two base camps. Attempts were made to obtain a minimum of 20 specimens of other species. Collections were by seine or dipnet, and specimens were preserved in 10% formalin. For specimens identifiable to species, heads will be preserved separately for use in otolith analyses (see below).

Due to small stomach volumes of YOY fishes, percentage relative volume of stomach content categories was estimated using a modified point system (Hynes 1950), where relative percentage volume of each category was estimated with an ocular grid. For catostomids with relatively undifferentiated digestive tracts, the intestine anterior to the first loop towards the head was arbitrarily delimited as "stomach." Volume of food occupying this portion of the gut relative to its potential volume was subjectively estimated and assigned a percentage value from 0 (empty) to 100 (full). Hynes (1950) and Corbet (1961) both reported that estimation of relative volumes does not differ significantly from direct methods of quantification. Numbers of identifiable taxa in stomachs were enumerated. Diel variations in feeding behavior will be investigated for specimens < 30 mm using the larval trap sampling regime described above.

Annual Hoop Net Monitoring

Hoop nets (2-3 m long, 6.4 mm mesh, 1.0 m diameter of the largest hoop) were deployed at 13 standardized locations in the lower 1200 m during the May annual monitoring period (Hendrickson and Kubly 1990). Nets were run daily, and lengths and weights of captured fishes recorded to ± 1 mm total length and ± 1 g, respectively. Native species longer than 150 mm were injected with passive integrated transponders (PIT tags) prior to release.

C, mean flow 0.05 m/s, conductivity 7500 μ S, dissolved oxygen near 6.0 mg/l, pH 6.9, and secchi depth > 1 m.

Although initially all eggs appeared healthy in the hatching trays, within a day the eggs from the naturally ripe females (no injected females had yet been stripped) in Salt Trail Canyon were smaller in size, exhibited more clumping, and had a higher proportion of white (dead) eggs than those in the LCR. The size difference was possibly due to ionic differences between the two sites. By the third day, eggs from both naturally spawned and earlier injected females incubated at both sites appeared mostly dead and decaying. Remaining early spawned eggs and later spawned eggs from injected fish were transported to Bubbling Ponds, but all had disintegrated within a few days.

Preserved egg samples were examined microscopically, and no evidence of cell division was observed. Apparently fertilization never occurred or eggs died soon after fertilization. The cause(s) of the lack of success of the egg culture attempt is not specifically known at this time. Strongly suspect, however, is the quality of the sperm diluent used, which was obtained from Dexter National Fish Hatchery. No knowledge of its age or expiration date was available. Eggs immediately began clumping after addition of this agent.

Water quality could also have been a factor assuming eggs were fertilized. High levels of silt in the LCR and high conductivity in Salt Trail Canyon could have suffocated eggs or created ionic imbalances, respectively. However, chub larvae were collected from the LCR in late April and early May, indicating that a successful natural spawn in these conditions occurred. Chub also have successfully spawned during periods of base flow when conductivities approached those in Salt Trail Canyon.

Larval Fish Longitudinal Surveys

Larval fishes began appearing in longitudinal surveys of the LCR in late April. Although all samples have not yet been identified, a few humpback chub larvae 10-12 mm were first collected on April 30 at 2070 m above the mouth, and later sporadic collections were made along most of the length of the river in early May, although numbers were low. Other native fish larvae were also collected rarely during these same periods, consisting mostly of bluehead sucker protolarvae and some speckled dace. Only one larval flannelmouth sucker has yet been identified from this period.

The paucity of captures of larval fishes in May 1992 compared to May 1991 (Angradi et al. 1992) may be partly attributable to inefficient sampling caused by high discharge (Figure

4.1) and turbidity in 1992, but may also be due to a scarcity of larvae. Although not yet fully analyzed quantitatively, habitat availability measurements at base and above base flows ostensibly show that nearshore current velocities are substantially higher during flood events. This indicates that nearshore slow-velocity larval habitats do not migrate laterally in the entrenched LCR as discharge levels increase above base flow, and thus larval habitat availability is reduced. In addition, since larvae are primarily sight feeders (Braum 1978), sustained turbidity associated with elevated flows may result in starvation.

Larval Fish Movements

No larval fishes were captured in larval fish traps, but several juvenile chub and dace were collected. The lack of larval fish captures may be reflective of the scarcity of larval fishes during this time.

Drift net samples collected between November and May have not yet been analyzed, but high discharges during the early reproductive period covered in this report typically prevented sets much longer than several minutes in duration due to large volumes of materials collected. This feature and those previously discussed makes it unlikely that many larvae were captured by this method during these conditions.

Length-Frequency Analyses

Growth of the 1991 humpback chub cohort between November and May was nearly static according to seine and dip net captures (Figure 4.2). Mean length of this age class in November was 82 ± 2.0 mm (SE), increased to 85 ± 2.6 mm in December, and remained nearly constant at $86-88 \pm 0.9-1.6$ mm thereafter through May.

Mean lengths of mixed age speckled dace (*Rhynchichthys osculus*) collected by seining and dip-netting increased from 71 ± 3.4 mm in November to 78 ± 1.5 mm in January, after which mean lengths fell to between 69-73 mm (Figure 4.3). Part of this decrease was due to captures of early post-larvae in March and May. Speckled dace apparently are able to spawn periodically during colder months in thermally-constant tributary springs.

Captures of age-0 bluehead (*Pantosteus discobolus*) and flannelmouth (*Catostomus latipinnis*) suckers between November and May were rare, especially for the latter species. Bluehead lengths in December ranged between 40-135 mm ($n=5$), all likely age-0 fish produced from an extended spawn in May-July of 1991 and a secondary spawn in October following summer floods (Angradi et al. 1992). By May of 1992, the range in length of probable age-0

blueheads spanned 39-176 mm ($n=3$). The uncommonness of juvenile catches following what was considered the most numerous larval species present in 1991 may indicate movement out of the LCR, habitat use shifts away from nearshore habitats less susceptible to seining, or high levels of post-larval mortality.

A total of eight flannelmouth suckers were captured in seines and dipnets between November and May. Lengths ranged between 79-157 mm. The scarcity of juvenile flannelmouth catches was likely due to low absolute abundance in the LCR. Larvae of this species were also only rarely encountered during 1991 (Angradi et al. 1992).

Annual Hoop Net Monitoring

Comparatively few fishes were captured in standardized hoop net sets in the lower 1200 m of the LCR in May 1992. Only 325 fishes total were caught, but relative abundances were similar to previous years, with humpback chub comprising 55.7%, speckled dace 29.5%, flannelmouth sucker 7.1%, bluehead sucker 4.9%, and nonnatives 2.8%. Catch rates averaged 0.42 fish/12 h (Robinson and Clarkson 1992). The modified Schnabel mark-recapture population estimate for humpback chub greater than 150 mm in the lower 1200 m was 168 (95% C.I. 90-299) fish, obviously an artificial artifact of sampling error perhaps influenced by low catch rates.

Maximum downstream distance recorded for recaptured humpback chub was 200 m. Maximum upstream recapture distance was 1020 m, with means of 80.5 and 409.5 m, respectively. Mean absolute distance moved between chub recaptures was 178.2 m. Mean number of days at large between recaptures was 2.5 (Robinson and Clarkson 1992).

Water Quality

Longitudinal patterns of selected water quality parameters in January were similar to those observed in October (Figure 4.4; Angradi et al. 1992). pH was nearly identical to the pattern and levels observed in October, ranging from 6.0 in the Blue Spring outflow 21 km above the mouth to 7.6 near the confluence. Conductivity levels exhibited a trend identical to that observed in October, but at slightly lower levels, presumably due to dilution by upper basin runoff (Figure 4.1). Above base flow discharge during the January sampling also likely reduced overall levels of alkalinity below Blue Spring compared to October levels.

Conversely, dissolved oxygen and carbon dioxide levels were generally higher in January, which was probably attributable to the greater solubility of these gases at lower temperature.

It is not known at this time the cause of elevated carbon dioxide levels. Turbidity levels below the Blue Spring outflow exceeded 100 NTU at all sampling sites.

Literature Cited

- Altman, J. 1974. Observational study of behavior: sampling methods. *Behavior* 49:227-265.
- Angradi, T. R., R. W. Clarkson, D. A. Kinsolving, D. M. Kubly, and S. A. Morgensen. 1992. Glen Canyon Dam and the Colorado River: responses of the aquatic biota to dam operations. Prepared for the Bureau of Reclamation, Upper Colorado Region, Glen Canyon Environmental Studies, Flagstaff, AZ. Cooperative Agreement No. 9-FC-40-07940. Arizona Game and Fish Department, Phoenix, AZ. 155 pages.
- Bovee, K. D. 1981. A users guide to the instream flow incremental methodology. U. S. Fish and Wildlife Service Biological Services Program FWS/OBS-80/52.
- Braum, E. 1978. Ecological aspects of the survival of fish eggs, embryos and larvae. Pages 102-131 in Shelby D. Gerking, editor. *Ecology of Freshwater Fish Production*. John Wiley and Sons, New York.
- Brown, A. V., and M. L. Armstrong. 1985. Propensity to drift downstream among various species of fish. *Journal of Freshwater Ecology* 3:3-18.
- Christensen, K. M. 1985. The linear interval method for determining habitat selection of riparian wildlife species. USDA General Technical Report RM--120:101-104.
- Corbet, P. S. 1961. The food of non-cichlid fishes in the Lake Victoria basin with remarks on their evolution and adaptation to lacustrine conditions. *Proceedings of the Zoological Society of London* 136:1-101.
- Corbett, B.W., and P. M. Powles. 1986. Spawning and larval drift of sympatric walleyes and white suckers in an Ontario stream. *Transactions of the American Fisheries Society* 115:41-46.
- Culp, J. M., and N. E. Glozier. 1989. Experimental evaluation of a minnow trap for small lotic fish. *Hydrobiologia* 175:83-87.
- Floyd, K. B., R. D. Hoyt, and S. Timbrook. 1984. Chronology of appearance and habitat partitioning by stream larval fishes. *Transactions of the American Fisheries Society* 113:217-223.
- Hamman, R. L. 1982. Spawning and culture of humpback chub. *Progressive Fish-Culturist* 44(4):213-216.

- Harvey, B. C. 1987. Susceptibility of young-of-the-year fishes to downstream displacement by flooding. *Transactions of the American Fisheries Society* 116:851-855.
- Harvey, B. C. 1991. Interaction of abiotic and biotic factors influences larval fish survival in an Oklahoma, USA, stream. *Canadian Journal of Fisheries and Aquatic Sciences* 48:1476-1480.
- Hendrickson, D. A. and D. M. Kubly. 1990. Arizona Game and Fish Department Grand Canyon humpback chub and razorback sucker monitoring project 1990 scope of work. Report and permit proposal to Grand Canyon National Park, Grand Canyon, Arizona, and Navajo Fish and Wildlife Department, Window Rock, Arizona. Arizona Game and Fish Department, Phoenix, Arizona. 14 pages.
- Hynes, H. B. N. 1950. The food of freshwater sticklebacks (*Gasterosteus aculeatus* and *Pygosteus pungitius*), with a review of methods used in studies of the food of fishes. *Journal of Animal Ecology* 19:35-58.
- Hynes, H. B. N. 1970. *The Ecology of Running Waters*. University of Toronto Press, Toronto. 555 pp.
- Lane, E. W. 1947. Report of the Subcommittee on Sediment Terminology. *Transactions of American Geophysics Union* 28:936-938.
- Manly, B. F. J. 1974. A model for certain types of selection experiments. *Biometrics* 30:281-294.
- Minckley, W. L. 1973. *Fishes of Arizona*. Arizona Game and Fish Department, Phoenix. 293 pp.
- Robinson, A. T., and R. W. Clarkson. 1992. Annual spring monitoring of humpback chub (*Gila cypha*) populations in the Little Colorado River, Grand Canyon, Arizona, 1987-1992. Draft Endangered Species Act Section 6, Project E5-2 Final Report to be submitted to U. S. Fish and Wildlife Service, Albuquerque, New Mexico. Arizona Game and Fish Department, Phoenix. 19 p.
- Snyder, D. E. 1981. Contributions to a guide to the cypriniform fish larvae of the upper Colorado River system in Colorado. Bureau of Land Management Biological Sciences Series No. 3:1-81.
- Snyder, D. E. 1990. Fish larvae--ecologically distinct organisms. Pages 20-23 in M. B. Bains, editor. *Ecology and assessment of warmwater streams: workshop synopsis*. U.S. Fish and Wildlife Service Biological Report 90(5).

- Thomas, D. L., and E. J. Taylor. 1990. Study designs and tests for comparing resource use and availability. *Journal of Wildlife Management* 54:322-330.
- Tyus, H. M. and G. B. Haines. 1991. Distribution, habitat use, and growth of age-0 Colorado squawfish in the Green River Basin, Colorado and Utah. *Transactions of the American Fisheries Society* 120:79-89.

TABLE 4.1.--Substrate categories used in AGFD habitat studies in the Little Colorado River. Size designations are based on grouped substrate classes of Lane (1947). Other categories were developed to account for unique substrates of the LCR.

CATEGORY	DEFINITION
Boulders	4.096-0.256 m dia
Cobbles (rubble)	256-64 mm dia
Gravel	64-2 mm dia
Sand	2-0.062 mm dia
Silt	62-4 μ m dia
Clay	4-0.24 μ m dia
Bedrock	> 4.096 m dia
Calcium Carbonate	Unconsolidated fine floc
Tufa	Consolidated calcium carbonate
Detritus	Decomposing organic material

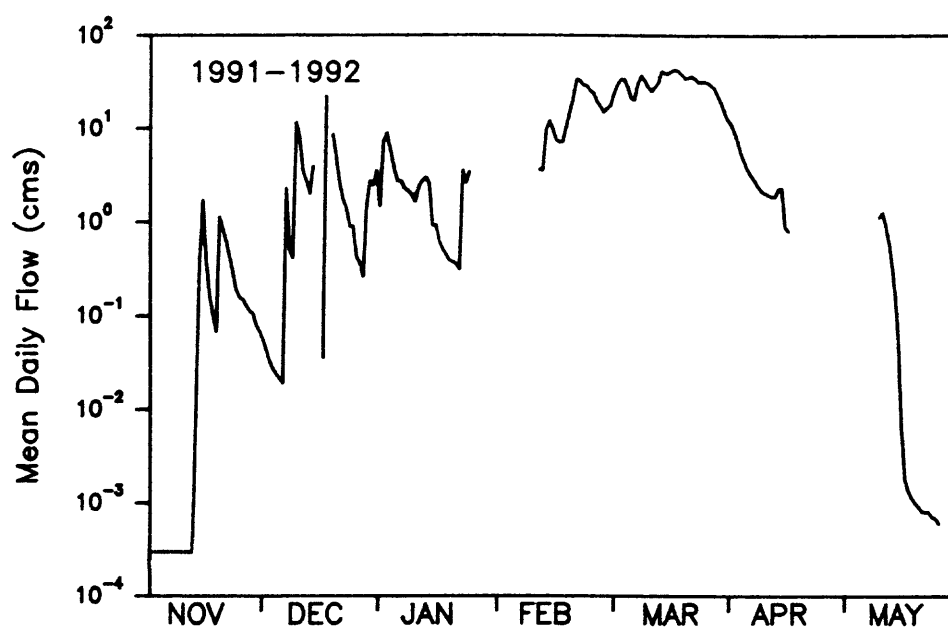


FIGURE 4.1.-Mean daily discharge (cubic meters per second [cms]) of the Little Colorado River at Cameron, AZ, November 1, 1991-May 30, 1992. Blank portions of the graph are missing values.

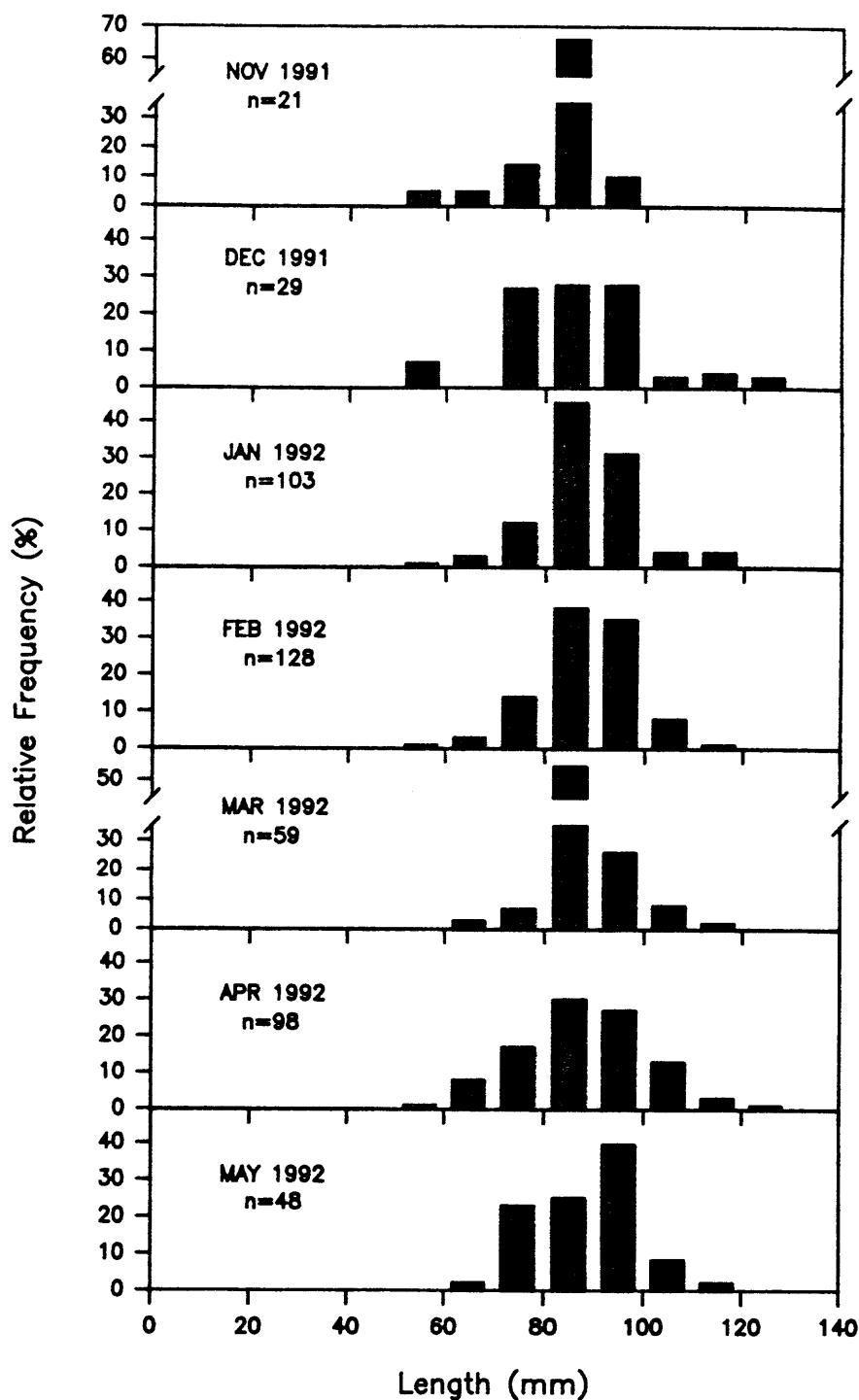


FIGURE 4.2.-Length-frequency distribution for putative age-0 humpback chub collected by seine and dip net in the Little Colorado River, November 1991-May 1992.

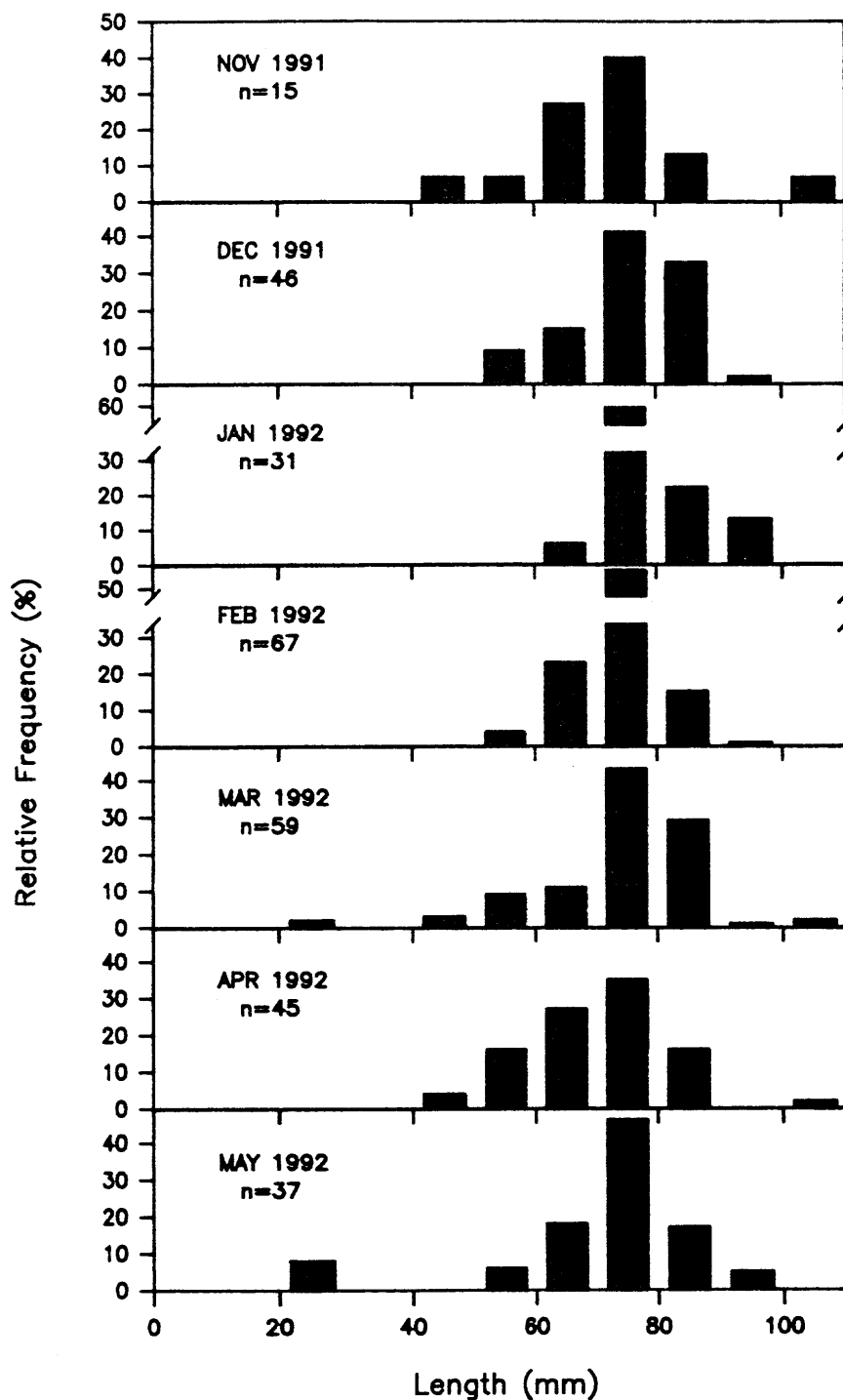


FIGURE 4.3.-Length-frequency distribution for speckled dace of mixed age collected by seine and dip net in the Little Colorado River, November 1991-May 1992.

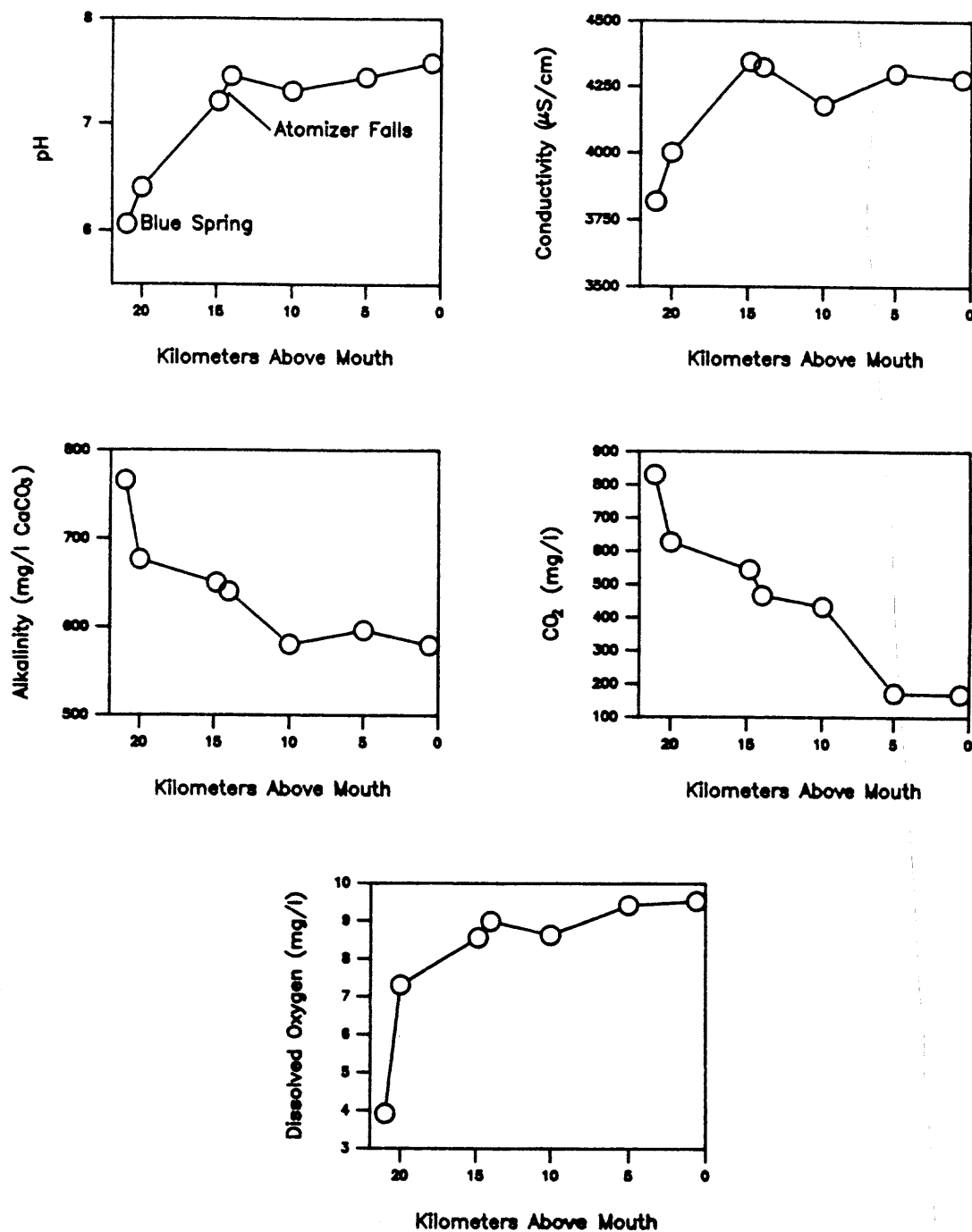


FIGURE 4.4.-Longitudinal patterns of selected water quality parameters from the Little Colorado River, January 1992.

5. Trout Studies - Lee's Ferry Reach

Terry R. Gamble

This chapter updates research on rainbow trout (*Oncorhynchus mykiss*) in the Lee's Ferry reach from November 1991 through May 1992. This period continued research activities as described by Angradi et al. (1992). Methods were refined to improve sampling techniques and sample more areas to evaluate the effectiveness of the new interim flow regime. Some methods have been refined so that they could effectively be integrated into a long term monitoring plan. In particular, methods for the age, growth, and population dynamics study are described as they were not included in the previous report. Only methods that differ from the previous report are described here.

Trout Spawning

The trout spawning experimental design was originally predicated on the assumption that most spawning occurred on cobble bars. Direct observation of fish during the 1990-1991 spawning season indicated that alternate habitats were used for spawning. We observed that during the 1990-1991 spawning period the majority of cobble bars were not used by spawning trout. Trout were observed using other areas of the river for spawning; chiefly shoreline areas that seemed to offer an acceptable range of substrate size and current velocity. Trout were also observed using deeper water spawning areas.

Methods have been changed in order to sample those habitats where trout were observed spawning in addition to cobble bars. This increased habitat usage greatly exacerbates the problem of quantifying suitable spawning areas and measuring the areal gain or loss due to fluctuating flows. Quantifying deep water spawning areas is problematical due to limitations in sampling techniques which includes SCUBA. An absolute areal measurement of spawning habitat in the reach may be an unobtainable goal. Methods were changed to allow integration into a long term monitoring plan. The approach which we are using provides a relative index that provides for specific sampling sites that could be sampled over a period of years to measure spawning success against changes in yearly flow regimes.

Efforts to measure substrate particle size, degree of embeddedness, current velocities, and water depth over the study plots in order to quantify suitable spawning habitat for trout in this reach remain substantially unchanged from the previous report. Previously cobble bars located at river miles -4.0, -6.1, -8.9, and -14.0 were studied. No spawning was observed on -14.0 mile bar and negligible spawning occurred at -6.1 mile bar. Due to manpower demands,

desire for increased sampling intensity, and the need to establish long term monitoring, the study of traditional cobble bars has been limited to -4.0 mile bar. This bar is the largest and most significant for spawning in the reach. A study site at -13.5 mile was also established to represent habitat located off cobble bars. The site is an area of shoreline that was used extensively by trout for spawning this year and in 1990-1991. The new study site was surveyed, and substrate particle size and imbeddedness were measured in January and March of 1992 duplicating the methodology used last year on -4.0 mile bar. Future analysis will evaluate if significant differences between these sample sites exist.

Velocity Measurements: In March of 1992 extensive current velocity readings were taken at each study site. Velocity measurements were taken at 2 or 4 meter intervals along bearings from known survey stakes. Distance from the stake to the edge of water (EOW) and to each station along the transect was recorded and a flow reading was taken at 10 cm from the bottom at each station that was inundated to at least that depth. Ten centimeters from the bottom was chosen as we believe this would represent the area utilized by fish searching for potential redd sites. When the change in bottom relief was not great, readings were taken at 4 meter intervals. In areas of greater change, 2 meter intervals were used. Readings were taken to a distance of 100 meters from the shore (-4.0 mile bar) or to where the *Cladophora* became too thick and tall to get accurate readings (-13.5 mile bar). This *Cladophora* line was well past where any redd building activity had been observed. Velocities have been taken at approximately 7000 cfs and at approximately 12000 cfs. It is intended that this procedure be duplicated for two additional flow regimes. To date, all measurements have been obtained by wading from shore. Greater flow volumes and the corresponding deeper water will require modified methods. We expect to experiment with deploying the flow meter from a boat or using a diver to get the measurements in the deep, fast water. This will take some experimentation and will be practiced before actually setting up field work.

Redd Placement: Originally, redd placement was determined at known spawning bars through out the spawning season. Redds were identified, counted, and their positions located by means of intersecting two compass bearings taken from known locations (surveyed stakes). An inherent problem with this method was over estimating the number of redds because of the inability to distinguish between previously counted and new redds during each consecutive survey of the spawning bars. Colored rocks were placed in redds when first counted, but the fish and fishermen removed them between sampling periods.

In March of 1992 a new method was adopted. To avoid duplicate redd counts, and more effectively integrate with a long range monitoring plan, it was decided to locate redds only once a year (a "snapshot in time" of redd placement). This was done near the peak of spawning to allow for placement behavior that might be based on relative positions of other redds and to assure a reasonable number of redds. These redds were located using the same compass bearing intercept method as last year. This survey method can be continued through the years and accurate index of spawning success can be developed.

Integration: Each of the data sets resulting from these new methods will be in the form of a matrix (x,y values) that describe the bar in terms of that variable. It is the intention of this design that each of these matrixes can be overlaid (along with the spawning gravel data set) onto surveyed elevations of the appropriate spawning bar. The result will be an x,y,z set of coordinates that will allow analysis to determine if relationships exist between elevation, sediment size distribution, flow, and redd placement. If such analysis yields positive relationships, it could be a useful tool in predicting and quantifying potential spawning areas in Glen Canyon.

Trout Stranding

Surveys to determine the effect of fluctuating flows on the stranding of adult trout and trout fry continued, using the same methodology as last year. The only significant change from last year is that 3 of the sites are no longer surveyed due to their being continually inundated under the new interim flow regime. One additional site, located at river mile -0.5 is no longer surveyed owing to difficulties in getting equipment and personnel safely to the site.

Age, Growth, and Population Dynamics

Electrofishing the Lee's Ferry reach on a quarterly or semiannual basis for a number of years. These data have been used to generate length frequency histograms and relative weight comparisons. In 1990, electrofishing efforts methodologies were as described by Maddux et al. (1987). In May 1991, it was decided that a more consistent, standardized methodology would be required to better assess changes in catch per unit effort (CPUE). Further a more structured protocol would allow a more accurate population estimate to be made.

Standardization: Field techniques have been standardized to ensure comparable result between sampling efforts. The current protocol requires sampling using the same equipment, personnel, flow regimes, and holding tanks, to the extent to minimize differences interjected by

variable methods. Scales are calibrated before each effort. Since May of 1991, only the Bureau of Reclamation's shocking boat has been used in order to ensure consistency in the gear. Mike Yard or Alan Hayden (Bureau of Reclamation employees) have been the only boat operators used and both are well versed in the experimental design. Manpower needs have been met largely with trained fisheries technicians who have had specific training in the procedures of this study. Data collection and data base management have also been standardized to ensure accurate and comparable data.

We currently are sampling on a quarterly basis, with electrofishing bouts scheduled at the same times each year. During these efforts, 15 sites, representing all habitats present in the reach, are surveyed at similar flow regimes.

Experimental design: Direct observation suggested a variety of habitats and microhabitats present in this reach. Particularly evident is a longitudinal difference in the character of the river from the upstream section to the downstream portion. We chose to map the river using basic habitat types for comparison. Towards this end, the river was broken down into 3 main habitat types which are roughly analogous to the classic riffle, run, and pool. During extremely low flows, (1000cfs-5000cfs) these habitats look like the classic riffle, run, and pool but this visual distinction is largely lost at higher flows.

Habitat #1 is roughly analogous to the riffle and comprises those portions of the reach associated with the cobble bars. Characteristics of this habitat: 1) higher current velocities and shallower water 2) steeper longitudinal gradient 3) greater "tidal" effect from fluctuating flows 4) cobble or boulder substrate with less sedimentation and 5) large portions of continually inundated substrate covered with *Cladophora*. Habitat #1 is further subdivided as the inside and outside of the river bend. The inside of the bend is typically shallower and is the cobble "beach" area of the habitat. The outside of the bend is typically the deepest portion with boulders being the primary substrate.

Habitat #2 is roughly analogous to the run and it is an intermediate habitat between habitats #1 and #3. It resembles habitat #1 but has slower current velocities. Characteristics of habitat #2 are: 1) intermediate current velocities with some deeper water 2) intermediate longitudinal gradient 3) largely sand substrate with some silt 4) less "tidal" fluctuations with the areas of fluctuation typically being sand beaches 5) some rooted vegetation but typically a "shifting sand" substrate which discourages vegetation. Like habitat #1, habitat #2 is further subdivided into the inside and out side of river bends. The outside bend of habitat #2 can

closely resemble habitat #1 with deep (but slower moving) water and boulder substrate while the inside of the bend is typically a sand beach.

Habitat #3 is roughly analogous to the pool. Characteristics of this habitat: 1) deeper water with slower current velocities 2) shallow longitudinal gradient 3) almost no "tidal" effect due to fluctuating flows 4) sand or silt substrate 5) area of greatest sedimentation 6) large rooted aquatic vegetation is prevalent along the margins providing excellent cover for macroinvertebrates and smaller fish 7) some of the habitat is so deep that it's characteristics are largely unknown. The river banks of habitat #3 are varied but there is little difference between the inside and outside of bends so there is no reason to further subdivide them.

The river was thus divided into these five habitat types. Habitat #1 comprises 34.4% of the reach (17.2% inside of bends and 17.2% outside of bends). Habitat #2 coincidentally also comprises 34.4% of the reach (again 17.2% inside and 17.2% outside) Habitat #3 comprises 31.1% of the reach and needs no further breakdown.

Within each habitat type, the river was broken down into as many 640 meter stretches as possible. A random number table was used to select 3 sites within each habitat. Site length, (640 meters) approximates 0.4 miles and was judged to be the smallest practical size for consistent electrofishing.

Angradi, T. R., R. W. Clarkson, D. A. Kinsolving, D. M. Kubly, and S. A. Morgensen. 1992. Glen Canyon Dam and the Colorado River: responses of the aquatic biota to dam operations. Prepared for the Bureau of Reclamation, Upper Colorado Region, Glen Canyon Environmental Studies, Flagstaff, AZ. Cooperative Agreement No. 9-FC-40-07940. Arizona Game and Fish Department, Phoenix, AZ. 155 pages.

Maddux, H. R., D. M. Kubly, J. C. deVos, Jr., W. R. Persons, R. Staedicke, and R. L. Wright. 1987. Effects of varied flow regimes on aquatic resources of Glen and Grand Canyons. Final Report to GCES, Arizona Game and Fish Department. 291 pages.